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CLINICAL AND EXPERIMENTAL

A COMPARISON OF THE AGGLUTININ AND ANTITOXIN CONTENT OF ANTIDYSENTERIC SERUMS*

BY ELLA M. A. ENLOWS, PH.D., AND S. C. BROOKS, PH.D., WASHINGTON, D. C.

THE agglutination technic† for testing the efficiency of antidysenteric serum has been used in this laboratory for a number of years.

Three isolations of the Shiga type (*Eberthella dysenteriae* Shiga) and two of the Flexner type (*Eberthella paradysenteriae*) are employed as antigens. As controls a typhoid suspension (*Eberthella typhi*), a polyvalent serum of known titer, and normal horse serum are used. These antigens are prepared in formalinized saline solutions, stored at 15° C. and frequently are found to vary but little for a considerable period of time (two to four years). Since it has been shown by a number of investigators and by our own experiments that antidysenteric serum when properly prepared contains substances capable of neutralizing the toxin of certain dysenteric types, the question has arisen as to the value of continuing the agglutination test as a measure of efficiency of this antiserum.

Castellani (1901), Inomata (1913), and Baldwin and Rhoades (1925) have briefly discussed the parallelism of agglutinins and protective substances.

It is recognized that the mechanism of immunity when broadly conceived comprehends very much more than the neutralization of a bacterial toxin by an antitoxin, but clinical experience with some serums (e.g., diphtheria antitoxin) has shown that we may use this phenomenon as an index of the efficiency of certain specific therapeutic or prophylactic serums. Nor can we dispute the evidence as to the efficiency index of antiserums secured from the

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†(Acél and Acél-Vecsei, 1924; Aoki, 1923; Arkwright, 1920; Castellani, 1901; Dopter, 1905; Dreyer, 1904 et seq.; Duhot and Damade 1919; Fabry, 1921; Gardner, 1923; Hausherr, 1921; Hirzfeld, Przesmycki, Seydel, and Sierkowski, 1922; Loewenthal, 1920; McLeod and Ritchie, 1920.)

protection of certain animals against lethal doses of the specific living organisms, and this, despite the fact that at times no definite antitoxin, agglutinin, nor bacteriolysin may be demonstrated.

The agglutinin content of the serum of immune or treated animals has been the characteristic most frequently determined. Perhaps this has been due largely to its ease of demonstration and to the failure to find other antibodies by the methods at our disposal. Our knowledge has advanced to a point where it is possible for us to separate specific major agglutinins from the so-called "normal," or minor and group agglutinins (agglutinin-absorption technic). That is, the agglutination technic undoubtedly is efficient in demonstrating animal reaction to certain administered bacterial organisms. Perhaps then it is well not to be too hasty in discarding altogether so useful a technic. It does show that certain treatment, with particular organisms, has been applied to the animals from which the serum has been secured, and it is necessary to know this in the case of a polyvalent serum such as is manufactured for use as an antidysenteric serum. While in the case of the Shiga type, we can demonstrate specific antitoxic substances in these polyvalent serums, it is difficult to do this in the case of the paradysentery strains since only weak toxins are formed by these types. Therefore, by the use of the agglutination technic in testing these serums we may gain some information as to the nature of the treatment used for their preparation.

In general, the comparisons between agglutinin and antitoxic content of antidysenteric serum to which reference is made in the literature appear to show that the two antibodies bear no close relation to each other.

Very few detailed results are published and most of these are based upon very meager data.*

EXPERIMENTAL DATA

During the past four years all of the commercial antidysenteric serums received at the Hygienic Laboratory have been tested both for their agglutinin and antitoxic content. Since no standard antitoxic serum was available at the beginning of this work, the comparative antitoxic value of the commercial serums is not well shown. The main fact to be demonstrated by the data presented is that the methods employed in the preparation of antidysenteric serums by the commercial laboratories are such that antitoxin is formed as well as agglutinins. In very few instances was there found a total lack of antitoxin as demonstrated by the methods used.

Among all of the bacterial toxins so far studied there appears to be none approaching that of *Eberthella dysenteriae* Shiga in irregularity of action upon animal inoculation. This fact is made exceedingly convincing by a brief glance at the pages and pages of published information on the toxins of this organism and its close relatives. Some investigators have continued their studies on these toxins over a long period of years (e.g., Doerr et al.), and, still feel that there is uncertainty as to their mode of action. We have, therefore, expected the irregularities shown in the tables submitted and have

*Sudmersen and Eagleton (1921); Douglas (1921); Hirzfeld, Przesmycki, Seydel and Sierskowski (1922).

endeavored to meet some of them by using varying doses of serum against a constant multiple of the M.L.D., and by the use of a dried toxin which we have found to be stable for a period of over six years. This procedure, while assuring us a fair degree of certainty as to the toxin used does not, of course, eliminate differences between individual animals. Throughout our work on this problem painstaking necropsies of all animal fatalities have been performed (combined with cultural methods). There is no possible way of avoiding this, if accuracy is desired, when the animals used are rabbits or albino mice. With the latter, mouse typhoid *(Salmonella typhi murium)* is responsible for many of the irregularities, especially at certain seasons of the year.

The irregularities are not so marked when rabbits are used as the test animal. It has been very difficult to obtain mice free of mouse typhoid (Webster, 1922), hence, almost always variable numbers of animals had to be considered in computing the protection percentage. Naturally had more animals been used for each test the variability would have been reduced. Without discussing further the nature of the toxins produced by the growth of *Eberthella dysenteriae* Shiga, we will merely state that we have been unable to separate our toxins in the clear-cut style described by Olitsky and Kligler (1920). Some toxins showed, in a majority of tests, decided effects upon either the central nervous system or the intestinal tract and these have been designated "neurotoxin" and "enterotoxin," respectively; however, lesions were nearly always produced in both the central nervous system and intestinal tract in a certain number of tests.

DISCUSSION OF DATA

It is the probable therapeutic value of antidysenteric serums which we attempt to determine. To do this both the agglutinin and antitoxin titrations have been used. The former yields for the same expenditure of time and money the more definite results, and if it could be shown to give correct information as to the probable therapeutic values of different serums it would naturally be preferred.

But since the toxic substances produced by the growth of the Shiga type of dysentery bacilli are apparently the chief cause of the lesions occurring in dysentery of this type, one is inclined on theoretic grounds to prefer antitoxin titration as a measure of the probable therapeutic value of antidysenteric serums. The possible existence of more than one toxin is a complicating factor, since any given technic of antitoxin titration measures the power of the serum to protect against one or the other toxin, or some one definite mixture of the two. The problem then resolves itself into a study of the following questions: (1) are agglutinin and antitoxin titrations correlated? (2) are antineurotoxic and antienterotoxic titers correlated? (3) are any of these correlated with therapeutic efficacy?

The data available are not extensive enough to yield a very satisfactory answer to these questions. But since these data are fairly extensive it has seemed worth while to make more than a cursory analysis of them. We publish the results of this analysis, fully conscious that the application of statis-

TABLE I

THE AGGLUTININ TITER OF SERUM FROM GOATS INJECTED SUBCUTANEOUSLY WITH KILLED AUTOLYSED CULTURES OF EBERTHELLA DYSENTERIAE SHIGA, R. I. 100, OVER A PERIOD OF THREE AND ONE HALF MONTHS, AND THE PROTECTIVE POWER OF THE SAME SERUMS WHEN INJECTED INTO MICE TOGETHER WITH TOXINS PREPARED BY PRECIPITATION FROM FILTRATES OF BOUILLON CULTURES (NEUROTOXIN AND ENTEROTOXIN) FOURTEEN DAYS OLD OF THE SAME STRAIN. (R. I. 100).*

SERUM	HIGHEST SERUM DILUTION FOR COMPLETE AGGLUTINATION WITH EBERTHELLA DYSENTERIAE						PER CENT OF MICE SURVIVING AFTER INJECTION OF SERUM-TOXIN MIXTURE	
66	SHIGA A RI 100	SHIGA B RI 1145	SHIGA 5 HL 73	FLEXNER (HARRIS) HL 115	FLEXNER (HISSY) HL 116	TYPHOID (RAWLINGS) HL 24	NEUROTOXIN	ENTEROTOXIN
None (normal saline)	0	0	0	0	0	0	25	0
Normal horse serum	0	200	0	0	0	0	0	0
Polyvalent Antidysenteric DI	1600	6400	3200	6400	400	0	60	70
Goat 1	1600	3200	800	0	0	0	89	72
Goat 2	1600	3200	800	0	0	0	86	89
Goat 3	200	200	0	0	0	0	100	72
Goat 4	1600	1600	400	0	0	0	12	67

*The serums from each goat were tested before treatment and found not to agglutinate any of the strains listed.

tical methods to so small a group of cases may prove misleading, and that it is justifiable only because of the improbability that more adequate data will be available in the near future.

Table I is not susceptible to statistical analysis, but offers some evidence as to the relationship between agglutinin and antitoxin titers. In it are the results of the agglutination and antitoxin tests with four goat serums of which Nos. 1 and 2 showed but very little difference in agglutinin content, Goat No. 2 showing a slightly higher titer with the culture which was used for its immunization. Goat 4 was almost equal to Nos. 1 and 2 against the homologous culture (Shiga 100) but dropped down in titer against H.L. 73. Goat 3 gave a low agglutinin titer. The toxin tests were run only against Shiga R.I. 100. Goat 3 showed a very good antitoxin content against both of the toxins in spite of its low agglutinin titer. Goat 4 showed very little antineurotoxin content, but fair antienterotoxin content.

From these data we may give a partial answer to the first two questions: agglutinin and antitoxin titers do not necessarily run parallel, and the relative protective powers of different serums when tested against that toxin designated as neurotoxin are not necessarily the same as when tested against that considered as enterotoxin.

TABLE II

COMPARISON OF THE AGGLUTININ TITER AND ANTITOXIN CONTENT OF 17 COMMERCIAL SERUMS, TOGETHER WITH SIMILAR DATA FOR A POLYVALENT ANTIDYSENTERY SERUM (D1). SERUMS 0.1 TO 0.005 C.C. TOXIN, (SHIGA R. I. 5, H. L. 73): 8 M.L.D.

COMMERCIAL SERUM NO.	AGGLUTINATION		ANTITOXIN			
	HIGHEST SERUM DILUTION FOR COMPLETE OR NEARLY COMPLETE AGGLUTINATION		CONTROL		COMMERCIAL	
	CONTROL SERUM	COMMERCIAL SERUM	NO. OF MICE	SURVIVALS IN PER CENT	NO. OF MICE	SURVIVALS IN PER CENT
30	3200	1600	--	--	9	88
30	3200	1600	--	--	10	80
31	3200	3200	--	--	5	60
32	3200	3200	5	60	6	83
33	3200	3200	5	60	7	85
34a	3200	1600	6	83	7	100
34b	3200	3200	3	66	4	75
35	3200	3200	7	100	8	75
36*	3200	0	3	66	6	0
37	3200	1600	3	66	1	100
38	3200	3200	3	66	5	40
39	3200	3200	3	66	5	80
40	1600	1600	3	66	5	60
41	1600	1600	3	66	3	0
42	1600	800	3	66	4	75
43	1600	3200	3	66	5	0
44	1600	3200	3	66	5	60

*Foreign sample.

Tables II to IV give a comparison of the agglutinin and antitoxin titers of a series of commercial serums. In considering the agglutination data the variable titer of the control (polyvalent antidysenteric) serum will be observed. This is due to the use of different antigens. It is constant, of course, for each individual lot of antigen. Nonspecific clumping or settling out is to be constantly guarded against in dysentery bacilli suspensions and for this

TABLE III

SAME SERUMS SHOWN IN TABLE II, BUT TESTED AGAINST TOXIN NO. 55 STYLED "ENTEROTOXIN" SINCE IT SHOWED GREATER TENDENCY TO PRODUCE LESIONS IN THE LARGE INTESTINE. SERUM: 0.1 TO 0.005 C.C. TOXINS 8 TO 10 M.L.D.

COMMERCIAL SERUM NO.	AGGLUTINATION		ANTITOXIN			
	HIGHEST SERUM DILUTION FOR COMPLETE OR NEARLY COMPLETE AGGLUTINATION		CONTROL		COMMERCIAL	
	CONTROL	COMMERCIAL	NO. OF MICE	SURVIVALS IN PER CENT	NO. OF MICE	SURVIVALS IN PER CENT
30	3200	1600	--	--	8	75
30	3200	1600	--	--	5	40
31	6400	3200	--	--	5	80
32	3200	3200	7	100	8	75
33	3200	3200	7	100	8	50
34a	3200	1600	3	100	4	100
35	3200	3200	4	75	3	66
34b	3200	3200	6	50	5	80
36*	3200	0	6	50	4	0
37	3200	1600	2	50	5	20
38	3200	3200	2	50	2	0
39	3200	3200	2	50	4	50
40	1600	1600	3	100	4	50
41	1600	1600	3	100	5	40
42	1600	800	3	100	5	20
43	1600	3200	3	100	6	50
44	1600	3200	3	100	6	0
45	3200	1600	3	100	3	66

*Foreign sample.

reason it sometimes seemed desirable to use an antigen giving a lower titer with the specific serum. Inspection of these tables will show numerous cases in which a high agglutinin titer accompanied high antitoxic content. But a number of exceptions will also be noted: serums 41 and 43 (Table II) have a high agglutinin titer but are deficient in antitoxin content while serum 42 (Table II) exhibits a much higher antitoxic content than would be expected on the basis of agglutinin titer.

A further insight into the extent of the correlation between agglutinin and antitoxin in titers may be gained by a somewhat more detailed analysis. For this purpose Tables II to IV are used.

For the purpose of this analysis we may in each table divide the serums into groups in which (1) the agglutinin titers of commercial and control serums are the same; (2) the commercial serum has the higher titer; and (3) the commercial serum has a lower titer than the control. For each of these groups we may calculate for both commercial and control serums the mean of the survivals in per cent and its probable error. If agglutinin and antitoxin titers are appreciably correlated the ratio of these two should be about one for group one, more than one for group two, and less than one for group three. The actual results are given in Table V, and show that only in the case of the data taken from Table IV is there probably a material positive correlation. Table II actually suggests that high agglutinin titer is associated with low antitoxin titer. Table IV is, however, more significant than the other two tables both because of the greater number of tests included, and because the antitoxic content was determined by tests of protective power for rabbits, which yield more consistent data.

TABLE IV

COMPARISON OF 30 SERUMS TESTED IN RABBITS AGAINST TOXIN NO. 55 (SERUMS NOS. 46, 47, 48 AND 49), TOXIN NO. 74 (SERUMS NOS. 50-64 INCLUSIVE), TOXIN NO. 83 (SERUMS NOS. 65-75, INCLUSIVE), MAY 22, 1923 TO JUNE, 1925. THE SERUM WAS TESTED IN DOSES OF 0.002, 0.001 AND 0.0005 C.C. AGAINST 10 M.L.D.

SERUM NO.	AGGLUTINATION		ANTITOXIN			
	HIGHEST DILUTION OF SERUM FOR COMPLETE OR NEARLY COMPLETE AGGLUTINATION		CONTROL		COMMERCIAL	
	CONTROL	COMMERCIAL	NO. OF RABBITS	SURVIVALS IN PER CENT	NO. OF RABBITS	SURVIVALS IN PER CENT
46	800	800	4	50	4	75
47	800	6400	4	50	4	100
48	800	3200	5	40	5	40
49	1600	1600	4	75	4	75
50	6400	6400	4	100	4	75
51	1600	1600	4	50	4	50
52	1600	6400	4	50	4	25
53	1600	6400	4	50	4	75
54	1600	0	4	75	4	25
55	1600	0	4	75	4	25
56	1600	3200	4	75	4	75
57	3200	800	6	100	6	33
58	3200	400	6	100	6	33
59	3200	6400	6	100	6	66
60	6400	6400	6	66	6	66
61	6400	3200	6	66	6	50
62	6400	6400	6	66	6	66
63	6400	6400	6	66	6	50
64	6400	6400	6	66	6	16
65	3200	6400	6	100	6	66
66	3200	3200	6	100	6	50
67	6400	3200	6	66	6	66
68	6400	1600	6	66	6	66
69	6400	3200	6	66	6	0
70	6400	6400	6	66	6	16
71	6400	6400	6	66	6	83
72	6400	6400	6	66	6	0
73	6400	6400	6	66	6	16
74	6400	6400	6	66	6	33
75	6400	3200	6	66	6	83
76	6400	3200	6	100	6	16
77	6400	3200	6	100	6	16
79	6400	3200	6	100	6	33
80	6400	3200	6	100	6	16
81	6400	1600	6	100	6	33
82	6400	6400	6	100	6	66
83	6400	1600	6	100	6	16

It is obvious that where only two or three serums are involved the probable error is not only very high, but also has very little real significance.

This method of analysis leads us to conclude that there is a slight but very erratic correlation between agglutinin titer and antitoxic content.*

It seems reasonable to inquire whether the large discrepancies which sometimes occur between the two methods are not most often due to chance errors in the antitoxin titration. If this be so, the agglutinin titration may be a more accurate measure of antitoxic power than the antitoxin tests themselves, when these are conducted upon any practicable number of animals, and would also give a more reliable indication of the probable therapeutic value of a serum.

*An attempt was made to obtain more exact information by calculation of a correlation coefficient between relative agglutinin titer and antitoxic content. Although a small positive coefficient resulted, yet the paucity of the data make it seem inadvisable to do more than mention the fact. At least there is no contradiction of the expectation of a positive correlation.

It would be desirable to study the correlations between agglutinin titration, antitoxin tests, and therapeutic value. Only in this way could a final answer be given to the question of the relative value of the different tests.

TABLE V

A COMPARISON OF THE ANTITOXIC CONTENT WITH THE AGGLUTININ TITER OF COMMERCIAL ANTIDYSENTERIC SERUMS, EACH REFERRED TO ITS CORRESPONDING CONTROL. THREE GROUPS ARE DISTINGUISHED ACCORDING AS THEIR AGGLUTININ TITER IS EQUAL TO (=), GREATER THAN (>) OR LESS THAN (<) THAT OF THEIR CONTROLS. CALCULATED FROM TABLES II TO IV WITH THE OMMISSION OF THOSE SERUMS FOR WHICH EITHER AGGLUTININ TITER WAS EQUAL TO 0, OR FOR WHICH NO DATA ARE GIVEN AS TO THE ANTITOXIC CONTENT OF THE CONTROL SERUM

TABLE USED AS A BASIS	RATIO: AGGLUTININ TITER OF COMMERCIAL SERUM / AGGLUTININ TITER OF CONTROL SERUM	NUMBER OF SERUMS TESTED	MEANS OF SURVIVALS IN	
			CONTROL SERUM	COMMERCIAL SERUM
II	$= 1$	8	69 ± 3	65 ± 7
	> 1	2	67 ± 0	30 ± 21
	< 1	3	75 ± 4	92 ± 6
III	$= 1$	8	82 ± 6	54 ± 5
	> 1	2	25 ± 17	100 ± 0
	< 1	4	52 ± 13	88 ± 0
IV	$= 1$	15	70 ± 3	45 ± 5
	> 1	7	66 ± 6	64 ± 6
	< 1	13	87 ± 3	36 ± 5

CONCLUSIONS

The available data upon the relative agglutinating and antitoxic properties of a series of commercial antidysenteric serums have been examined with a view to determining whether agglutinin titer and protective power against neuro- and enterotoxins were equally indicative of the probable therapeutic value. This examination leads us to conclude:

1. That there is no necessary parallelism between agglutinin titer and antitoxic content as usually measured by protective power, nor between this protective power against neurotoxin as compared with enterotoxin; but:

2. That a slight positive correlation exists between agglutinin titer and antitoxic power so that high agglutinin titer tends to be accompanied by high protective power.

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OBSERVATIONS ON THE MORPHOLOGY AND MOTILITY OF FUSIFORM BACILLI*

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While the majority of investigators discredit the possibility of a genetic connection between the baeillus fusiformis dentium and the coarser type of spirochete accompanying it in normal and pathologic conditions, the question of their relationship is still frequently raised and their identity stated as a fact in some current textbooks. With the exception of the observations by Tunnicliff (1923), who has reported in detail the development of spiral forms in pure cultures of the bacillus, the evidence of the identity of the two organisms has been based upon the presence of occasional nonmotile spiral forms in cultures.

In view of the stimulating effect of fresh tissue on the multiplication of many varieties of spirochetes and of the observation by Tunnicliff that the addition of 1 to 2 drops of $\frac{N}{10}$ acetic acid or $\frac{N}{10}$ sodium hydroxide to the media favored the development of the spiral forms, it was deemed that a careful study of the morphology of the bacillus in media of varying hydrogen-ion concentration and containing fresh tissue would be of interest. Several workers have reported the use of fresh tissue in their cultural experiments but I have found no reference to a careful morphologic study of the bacilli made under such conditions.†

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†Recently Pratt (Jour. Inf. Dis. 1927, xli, 461) observed the growth of fusiform bacilli only, when cultured in serum water containing fresh tissue.

Tarozzi (1905)) failed to obtain a growth of the bacillus in a bouillon media containing fresh tissue which was incubated aerobically. He learned later, however, that the culture he had used was not viable. Schmamine (1912) succeeded in growing the bacillus according to the method of Tarozzi and observed that the forms were shorter than of those cultivated in serum. Brams, Pilot and Davis (1923) in their study on the flora of male smegma and Pilot and Brams (1923) culturing excised tonsils and adenoids cultivated the bacillus in ascites tissue broth in mixed culture. Pilot and Kanter (1923) obtained the organisms also in mixed culture from female smegma in ascites tissue agar, and Tunnicliff (1923) states the presence of fresh tissue did not appear to enhance the growth of the bacillus.

Source of Culture.—The strain of fusiform bacillus used in the present morphologic study, employing the media of varying hydrogen-ion concentration, was isolated from a case of chronic Vincent's angina by Mr. Austin Wakeford at the Thomas W. Evans Museum and Dental Institute, University of Pennsylvania. In serum agar tubes containing fresh tissue it frequently formed the typical colony extensions (Fig. 1) as noted by several observers including Lenkowiez (1903; 1906) who was the first to obtain a pure culture of the bacilli, Ellerman (1905) and Krumwiede and Pratt (1913).

An interesting colony formation of the bacilli from the pure culture was observed in a slide preparation incubated at 37° containing a small quantity of macerated fresh tissue in serum agar. The preparation should be practically free from air bubbles and the cover slip sealed with high melting point paraffin. The colonies consist mostly of organisms growing out in filaments from a central area. They increase gradually in size for forty-eight to seventy-two hours after incubation. Using a low per cent of agar, more isolated single forms appear but the granules are not usually visible under dark-field illumination during the period of active growth. Upon continuous observation under dark-field illumination, dividing forms in which the granules were visible were occasionally seen in the slide preparations incubated in a micro-stage incubator. The line of division extended diagonally from one side of a bacillus near one granule to the opposite side near another granule thus forming the characteristic pointed ends of the bacillus.

The change in the position of the divided bacilli explains some of the arrangements one frequently observes in preparations. In some instances, after the breaking apart, the outer end of one bacillus moved slowly to one side, its newly formed pointed end appearing to remain in contact with the other organism forming an angle. In other instances, after the separation, one bacillus slipped gradually beside the other forming the parallel arrangement.

Media Used.—Two series of fluid media were prepared in vaseline sealed tubes (20 by 1.5 cm.). The one series containing hormone bouillon at hydrogen-ion concentrations ranging from P_H 6.8 to 8.4 with and without fresh rabbit kidney tissue, with and without glucose (0.5 per cent) and with and without inactivated horse serum (4.1). In the other similar series Hartley's broth (1922) was substituted for the bouillon and chick embryo for the kidney tissue.

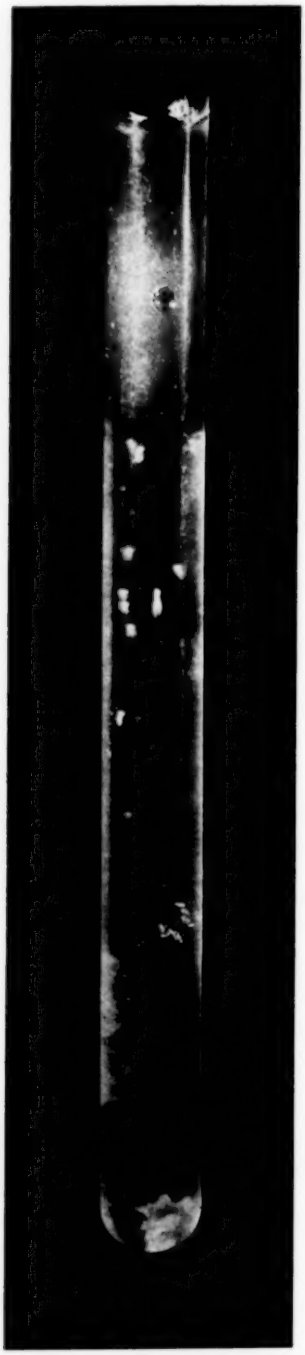

Fig. 1.—Characteristic colony extensions of the bacillus fusiformis dentium are shown in the upper isolated colonies.

Examination.—Two tubes of each variety of media were planted, one being examined almost daily for a period of two weeks, the other at longer intervals. All, however, were kept under observation for two months.

The following Zeiss microscopic equipment was used for all examinations: Siedentopf change, over condenser, objective (X), "Bitumi" binocular tube attachment with compensating eyepieces Kbi 12.5X; and "Tip up" type of micro lamp or arch light.

Preparations stained vitally with brilliant cresyl blue and smears stained with Giemsa, Gram, carbol gentian violet and dilute carbol fuchsin were also frequently made.

Result.—While the best growth of the bacilli occurred in both series of media with P_H 7.3 to 8.4 containing serum, glucose, and tissue, and the least in the media without these additional substances, a varying amount of growth with acid but no gas production took place in all.

Forms differing greatly in length and in number of visible granules were present and even though the granules cannot always be distinguished in the dark-field, they usually become visible when carefully stained with dilute carbol fuchsin.* An occasional long wavy form was seen but such forms are also common in cultures of other organisms which have a tendency to grow out in filaments. No indication of spirochetal forms developing from the granules could be detected at any time.

While the bacilli in the preceding pure culture study appeared to be slightly flexible they exhibited no motility. In this connection, however, I would like to report the interesting observation of very motile fusiform bacilli together with nonmotile forms in slide preparations made directly from human infections upon three occasions and in two instances in mixed culture for several generations. The material for the direct examinations was obtained from a discharging ear,† a pyorrhea alveolaris infection and a Vincent's angina case. Other organisms including cocci, the thicker motile type of spirochete and some of the thinner varieties were present in all three cases, also motile spirilla sputigenum in the last two and an anaerobic curved non-spore-bearing rod was isolated from the Vincent's angina, which will be described in a future report.

From the three cases referred to above, careful cultural experiments were made only with material obtained from the Vincent's infection. Cultures were made in deep tubes in various liquid and agar media with vaseline seal, with and without fresh tissue and in Schereschewsky's (1912) coagulated horse serum. A few nonmotile fusiform bacilli were seen in the mixed cultures in most of the media but motile fusiform bacilli were present only in the coaglulated serum media. The implantation in this media had been made on one side of the tube approximately two inches below the surface. No vaseline seal was used but the cotton stopper was paraffined. After four or five days' incubation the media above the inoculated area had become slightly

*I later observed that all the bacilli even in young cultures contained one or more granules which could be readily demonstrated with MacNeal's (1922) Tetrachrome stain using the phospate buffer of P_H 6.4 recommended by Scott and French (1924). For best results the preparation should be fixed with heat before staining.

†The laboratory examination of this case was reported in Arch. Derm. and Syph., 1927, xv, 678.

liquefied; the fluid spaces, viewed under dark-field illumination, were filled mostly with cocci, short motile bacilli and motile spirilla, while the more solid coagulated portions from the upper part of the medium contained the greater number of very motile Vincent's, microdentium and macrodentium types of spirochetes, nonmotile filaments varying in length, together with motile and nonmotile fusiform bacilli. Most of the filaments are evidently long forms of the fusiform bacilli since many contain granules with tetrachrome stain. In the lower part of the tube which showed no macroscopic change so far, only cocci could be seen.

Transplants using the coagulated particles were made to the same media in the same manner as in the primary cultures except that a vaseline seal was used.

Within seven days the area around the inoculated site had become light gray but not much liquefied. Numerous motile spirochetes, mostly of the macrodentium type and many motile and nonmotile fusiform bacilli together with other contaminants were again present in the upper coagulated portions. The media at the bottom of the tube again showing no macroscopic change contained, however, a good number of both motile and nonmotile fusiform bacilli together with only cocci. The hydrogen-ion concentration of the media at the bottom was P_H 7.5 and that at the top P_H 7.0.

Hoping to obtain a culture of the fusiform bacilli with possibly only the cocci contaminant, several subcultures from the material at the bottom of the tube were made to liquid serum media with fresh tissue and to the coagulated serum. Even after prolonged incubation, however, no macroscopic change occurred in the coagulated media and upon examination only a few cocci could be seen. A few nonmotile fusiform bacilli and cocci were present in the liquid tissue media. After the column of the coagulated medium containing the numerous contaminants at the top has been broken by inserting the capillary pipette for examination, the contaminants quickly spread and multiply throughout the tube, liquefying the media and producing a disagreeable odor.

The culture material for the second mixed culture of the motile fusiform bacilli was obtained from the spongy gum tissue surrounding an infected, decayed tooth. While the various types of spirochetes, nonmotile fusiform bacilli and motile spirilla were present, no motile fusiform bacilli were observable in the dark-field examination of the fresh preparation. In the cultural study, however, motile fusiform bacilli again appeared but only in the Scheresehewsky's media. Instead of attempting to purify the culture as in the first experiment, a small quantity of the culture from the upper part of the tube containing more of the contaminants together with material from the bottom containing the motile fusiform bacilli, was used in making the transplants. In this way the motile fusiform bacilli were cultivated for five generations but failed to grow in the sixth transplant.

The characteristic movement of the motile fusiform bacilli can be observed best in the cultures. It is usually a darting progressive movement in either direction and impresses one as being of a more purposeful nature than that exhibited by other varieties of motile organisms. It is of a much less

vibratory type than that of the spirillum sputigenum. Occasionally a bacillus and also a longer thread form with a slower yet distinctly characteristic motion are seen. In the coagulated media the motile bacillus is not as a rule in constant motion and when at rest it is indistinguishable from the nonmotile types. To one accustomed to the nonmotile forms only, the sudden movement of the motile forms through the field, piercing the coagulated particles with their sharp ends impresses one as being an unusual phenomenon. In preparations made from the coagulated serum cultures, motile fusiform bacilli were still present after two hours, but no definite tests were made to determine how long the motility would be retained in the slide preparation.

While some of the earlier writers claim to have observed varying degrees of motility of the bacilli in material taken directly from the seat of the disease, all of the more recent observers, with the exception of Stitt, have noted only nonmotile forms in fresh preparation and in mixed or pure cultures.

In view of my personal observations I will briefly review the statements of authors who have observed the bacilli to be motile. Bernheim (1898) describing the organisms present in ulceromembranous stomatitis states that the bacilli are motile like the spirochetes; Letulle (1900) observed the bacilli in Vincent's angina to be motile if mounted in saliva. Sobel and Herman (1901) report their observations to be in accordance with those of Letulle. Carnot and Fournier (1901) found the bacilli very motile if kept at the proper temperature. Graupner (1902) states the bacilli are very motile but relinquish their activity within twenty minutes in a slide preparation. He also demonstrated a flagella on either end and two on each side. Baron (1902) observed a slow swinging movement of the bacilli in a hanging drop. Vincent (1905) states that while the bacilli are usually nonmotile, he has observed some with slight motility in exudates but none in cultures. He also distinguishes the fusiform bacilli from the spirillum sputigenum because of the difference in their form, dimension, motility and staining reaction. Veszpremi (1907) found the bacilli to be actively motile in fresh preparations but not in cultures. Leiner (1907) noted a distinct and fairly active to and fro movement in a hanging drop. Plaut (1907) observed an unmistakable vacillating motion and also demonstrated flagella by staining. Costa (1909) describes in more detail than the previous observers the character of the motility of the bacillus found in lesions which have not been exposed to the air. Stitt (1923) states, "I have noted a sluggish but distinct motility when the material was mounted in saliva." The many other earlier writers and practically all the later investigators including Tunnicliff (1906), Larson and Baron (1913), Krumwiede and Pratt (1913) and many others, however, consider the bacillus nonmotile, Krumwiede and Pratt stating, "The motility of the spirochetes and the lack of motility of the fusiform bacillus form the strongest evidence against their identity." Various explanations have been offered to account for the motility observed by the earlier workers. Ellerman (1905) and Muhlens (1907) who cultivated both the motile spirilla sputigenum and the nonmotile fusiform bacilli in pure culture, thought that the motile bacilli observed by others were the spirilla. Larson and Baron, and Goadby (1922) express the possibility of the reported motility being due to the presence of the motile spirochetes. In

view of the handicaps in their methods of investigation and of the fact that the distinction between the motile spirillum and the fusiform bacillus was not generally recognized at that date, it is quite probable that mistaken identifications were made.

In the present study, however, with the dark-field equipment previously mentioned, both the morphology and characteristic motility of the bacilli could be quickly and beautifully demonstrated. While a long, coarse and frequently curved flagellum of the spirillum sputigenum is visible in some instances in the dark-field, no definite evidence of flagella could be seen on the motile fusiform bacilli. Several differences in the characteristics of individual strains of fusiform bacilli have thus far been generally recognized. The only variation noted by Krumweide and Pratt among the numerous strains they isolated was in the ability of some strains to ferment saccharose while others did not. Stitt recognizes two morphologic types, one which stains uniformly, the other showing the dots. Knorr (1922) describes three types differing among each other in colony formation, and Varney (1927) identified four serologic types of the bacilli and also distinguishes morphologic types. The motile bacilli I have observed are more of the longer than the shorter type which are most frequently present under normal conditions.

The real significance of the motile type, that is, whether it represents a distinct variety differing in cultural and possibly pathologic characters from the nonmotile type of the bacillus fusiformis dentium or whether the two are identical, the motility appearing only under certain conditions, must await further investigations. It would seem from my limited observations that the motile forms are more frequently present in more acute pathologic conditions since I have found no motile specimens in material taken from several normal oral cavities or extirpated tonsils, or in cultures in the Schereschewsky's media made from these sources.

In my future investigations I hope to obtain further cultures of the motile type and to study the relationship existing between the motile and nonmotile forms.

SUMMARY

1. No evidence of spirochetal forms was seen in a pure culture of fusiform bacilli grown anaerobically in media of varying hydrogen-ion concentration containing fresh tissue.

2. Colony formation and the division of fusiform bacilli were observed in a micro slide preparation.

3. Motile fusiform bacilli, together with nonmotile forms were found in material taken directly from pathologic conditions and in mixed cultures in Schereschewsky's coagulated horse serum media.

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THE SO-CALLED CHEMICAL TEST IN BLOOD FOR SEX DIFFERENTIATION*

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ACCUMULATED evidence has indicated that in the blood stream of animals may be found chemical units produced by the individual organs of internal secretion. On this basis, the chemical test for sex differentiation is not only an attractive idea but also very possible of solution.

A simplification of the Manoilov technic based on a study of the functions of the several reagents used by him has already been presented in a paper from this Laboratory.[1] It has been found necessary to devise a procedure for a more quantitative recording of the test reaction. In the original method, the end-result of a test on an extract of male origin, resulted in a colorless solution; of female origin, in solutions ranging in color from pink to red or violet.

In the above mentioned study it was shown that in the modified test the reaction color obtained was due to the formation of a "red acid" which could be prepared by adding a solution of potassium permanganate to an acid solution of pararosaniline hydrochloride. This "red acid" is insoluble in acids but soluble in alkali from which it can be reprecipitated by acid. Standards were prepared with this "red acid" which approximated very closely the colors

*From the Harriman Research Laboratory, The Roosevelt Hospital, New York. Received for publication, May 11, 1928.

obtained in the revised reaction and were similar also to the colors obtained in the original Manoilov reaction. These color standards were prepared in the following manner. To 4 c.c. of water in each of 12 tubes from 1 to 12 drops of a 0.1 per cent solution of the "red acid" in 1 per cent sodium hydroxide were added, followed by 10 drops of a 1 per cent solution of gelatin and 3 drops of 20 per cent sulphuric acid. The gelatin acts as a protective colloid which prevents the reprecipitation of the "red acid" by the sulphuric acid. The depth of color depends upon the amount of "red acid" present and was denoted by a number corresponding to the number of drops of "red acid" used. The standard tubes numbered 1 to 5 inclusive, ranging from colorless to but a faintly colored mixture may be taken to represent the male half of the scale; the tubes numbered 6 to 12, the last tube with a solution very deep brownish red in color, the female half of the scale.

Examination of Blood Samples.—The blood samples were tested as follows, in accordance with the revised technic: To 3 c.c. of a 5 per cent solution of citrated whole blood were added in succession 1 drop of a 1 per cent solution of pararosaniline hydrochloride in 95 per cent ethyl alcohol, 35 drops of 0.02 M. potassium permanganate (from a burette to get uniform drops), 3 drops of 20 per cent sulphuric acid and 10 drops of 2 per cent sodium thiosulphate. The samples of male blood were tested as well by the Manoilov reaction, Modification III)[2] and were found to closely parallel in color reaction the revised technic.

The summary of our findings is given in Table I. For both the male and female groups the samples as measured distribute themselves in approximately equal numbers between the male and female half of the scale. The sex of the donor, therefore, has no influence on this blood reaction. This conclusion is applicable with equal force to the original Manoilov reaction.

The examination of urine by the same procedure suggested itself. The following technic was adopted for the urine examination: To 3 c.c. of urine were added 2 drops of a 1 per cent solution of pararosaniline hydrochloride in

TABLE I

COLOR VALUE REVISED TEST	NUMBER OF TESTS								TOTAL TESTS
	AGE UP TO 30		AGE 31 TO 40		AGE 41 TO 50		AGE 51 UP		
	FEMALE	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE	MALE	
0	5		4		2				11
1	2								2
2	4		3		2		1	1	11
3	8		4	1	2		1		16
4	8	2	9	1	3	3	2		27
5	6	1	6	1	1	1			16
6	4		8	1	2				15
7	3	2	3	1	2				11
8	1		5	2	2	1	2		13
9			1	3		1		1	6
10	3		6		1		1		11
11			2						2
12	3		3			1	1	1	9
12+	2		3						5
Totals	49	4	57	10	17	7	8	3	155

95 per cent alcohol, 45 drops of 0.02 M. potassium permanganate (from a burette), 5 drops of 20 per cent sulphuric acid, and 10 drops of 2 per cent sodium thiosulphate.

The urines of more than 100 women were tested at different times, in many instances different samples were secured from the same individual. The color values were found to vary for the urine specimens of different individuals and also for different samples of the same individual. These variations covered the whole range of color of the standard tubes. Similar results were secured on male samples. Table II gives a typical result for one day on a male subject.

TABLE II

TIME	SPECIFIC GRAVITY	COLOR VALUE REVISED TEST
9 A.M.	1.030	10
10 "	1.027	3
11 "	1.016	0
12 NOON	1.019	0
1 P.M.	1.025	3
2 "	1.031	12
3 "	1.033	12+
4 "	1.030	12

The sex specificity of the Manoilov reaction has already been disavowed by several workers. Riddle and Reinhart,[3] based on a study of pigeon blood, read into this reaction a correlation between it and metabolic rate. Chemically considered, the procedure involves an oxidation process which in some samples of biologic fluid is permitted to go on to completion and others is inhibited at points along the line of completion. It is probable that no single chemical substance is required for playing the part of inhibitor in this reaction. It is unlikely that we are determining the presence of the same substances in our tests on urine and blood. The chemical reactions involved in this procedure are interesting and worthy of further study. Until such studies have been made and more specific knowledge of the chemistry of this reaction is available it will be unsafe to correlate with the findings in this reaction measures or features of major biologic reactions or characters.

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THE DISTRIBUTION OF CALCIUM IN JAUNDICED AND ACHOLIC DOGS*

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THE fact that a deficiency in calcium, in patients that are jaundiced, causes a prolonged coagulation time has long been observed. Lee and Vincent[1] have noted a prolonged coagulation time of the blood in patients who were jaundiced over a period of at least five weeks. These authors reduced the coagulation time of the blood in jaundiced patients in vitro as well as in vivo by the addition of calcium salts to the blood.

Grove and Vines[2] have shown that in the circulating blood, of the normal individual, calcium is present in two forms, namely, an ionized and a combined.

Klinke[3] believes that serum calcium is partly ionized (about three milligrams per hundred cubic centimeters) partly dissolved as a complex or molecular salt. The ionized calcium is ultrafiltrable and will pass through a collodion membrane.

We have then in the blood two forms of calcium, one a combined or nondiffusible and the other an uncombined ionized salt that is diffusible.

Kirk and King[4] report that in a series of seven cases of jaundiced patients the ultrafiltrable or diffusible calcium is below normal.

King and Stewart[5] state that in jaundiced dogs there is a 17 to 20 per cent increase in the serum calcium. King, Bigelow and Pearce[6] report a higher serum calcium content in the blood of jaundiced dogs than in the blood of normal dogs. Davidson and Emerson[7] report an increase in serum calcium following the injection of whole bile into the femoral vein of a dog.

We have a definite paradox; a lowering of diffusible calcium in jaundice, the fact that the addition of calcium salts to the blood of jaundiced patients will definitely shorten the coagulation time, and the reports of three authors who state there is a definite rise in serum calcium in jaundice. This may be explained as follows: In experimental work completed, unpublished data, we have shown that following ether anesthesia there is a very definite rise in serum calcium. King and Stewart[5] do not state whether blood was obtained for serum calcium before or after ether anesthesia. King, Bigelow, and Pearce[6] state that a week after ligation of the common duct their animals were bled to death under ether anesthesia. Davidson and Emerson[7] do not definitely state whether blood was obtained for serum calcium before or after ether anesthesia. However, having done this work we know that the blood for the serum calcium determinations was obtained following ether anes-

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thesia. This would account for the rise in serum calcium following the injection of whole bile and the rise in serum calcium in jaundice.

In all of the work, as reported in this paper, care was taken to obtain the blood for the calcium determinations before any anesthesia was given.

Kirk and King[4] report calcium in plasma in normal dogs under ether anesthesia was 8.5 mg. This is a higher figure than we obtained. However, here again the difference could easily be explained because of the anesthesia.

Cantarow, Dodak and Gordon[8] note quite a wide variation in the whole blood calcium in normal patients, the variation being 6.4 mg. to 9.6 mg. We noted the same variation in the whole blood calcium of normal dogs, the variation being 4.3 mg. to 7.0 mg.

King, Bigelow and Pearce[6] have noted that in dogs jaundiced a week there was a rise in the calcium content of the blood, kidneys, and feces with a decrease in the calcium content of the bone and heart. These findings do not correspond with those we obtained. The difference may be explained by the fact that their dogs were jaundiced only a week while our dogs averaged forty days each. In a week complete calcium balance would not be obtained.

Corley and Dennis[9] give the following results for the calcium content in normal dog's tissue: liver 26.4 mg., lungs 40.8 mg., and kidney 40.0 mg. These figures check very nicely with our results. We used their method for determining calcium in the tissues.

METHOD

Five normal dogs of as near the same weight as possible were obtained. They were placed on a mixed feed consisting of ground scraps from the hospital and containing meat, bread, bone, fruit, vegetables, and pastry. Their normal serum calcium, whole blood calcium, diffusible calcium, and spinal fluid calcium were obtained according to the methods of Clark and Collip[10] and Moritz.[11] On one dog samples of blood were taken on ten consecutive days at various times of the day and the serum calcium determined. These dogs were bled to death under ether anesthesia, care being taken to obtain the blood for the calcium determinations before the anesthesia was begun. In order to remove blood from the tissues, the dogs were perfused intravenously with a 0.6 per cent sodium chloride solution, the rate and volume being equal to the rate and volume of the blood passing out. Their organs were removed, dried to constant weight, and the calcium content determined according to the method devised by Corley and Dennia.[9]

Three other dogs were obtained of as near the same weight as possible. Their normal serum calcium, whole blood calcium, and diffusible calcium were determined. In each dog the common duct was ligated in two places, and a piece of the common duct excised between the two ligatures. Each week until the animals showed signs of severe intoxication, blood was taken for the determination of serum calcium, whole blood calcium and diffusible calcium. After the dogs had been jaundiced thirty-three, forty and forty-nine days respectively, they were bled to death under ether anesthesia, their tissues perfused, their organs removed, dried to constant weight, and the calcium content determined.

Three more dogs of as near the same weight as possible were obtained. Their normal serum calcium, whole blood calcium, and diffusible calcium were determined. In each dog a cholecystonephrostomy was done according to the method of Kapsinow, Engle and Harvey.[12] The gall bladder was sewed, after an opening had been made in the fundus, into the kidney pelvis. The common duct was ligated in two places and a piece of the common duct excised between the two ligatures. This produced a bile fistula, the bile passing out with the urine. Each week until the animals were killed, blood was taken for the determination of serum calcium, whole blood calcium, and diffusible calcium. At the end of seventy, eighty-four and eighty-four days respectively, the dogs were bled to death under ether anesthesia, their tissues perfused, their organs removed, dried to constant weight, and the calcium content determined.

In Dog 8, a jaundiced dog, and Dog 10, a bile fistula dog, x-rays of the pelvis and femurs of each animal were obtained at the beginning of the experiment and just before the animals were killed. The distance, voltage and milliampere seconds on the x-rays taken at the beginning of the experiment and at the completion of the experiment were the same. The films were developed the same length of time in each case. The densities of the pelvic bones and the femurs were obtained with a photometer.

To determine whether or not some of the calcium in the tissues might be washed out with the perfusing solution a normal dog was obtained and, under ether anesthesia, the renal artery and vein of one kidney were clamped off. The renal artery and vein on the other side were left intact. The dog was bled to death, the tissues were perfused, the two kidneys removed, dried to constant weight, and the calcium content determined.

The feces of two normal dogs, two jaundiced dogs, and two bile fistula dogs were obtained on three consecutive days. These feces were dried to constant weight and the calcium content determined according to the method of Corley and Denis.[9]

RESULTS

The diffusible calcium of the blood, the calcium content of the blood serum, whole blood, spinal fluid, brain, liver, spleen, muscle, bone, subcutaneous tissue, heart, thyroid, gall bladder, lung, kidney, and adrenal was determined in the blood and tissues of five normal dogs. The results are summarized in Table I.

The calcium content of the blood and tissues enumerated above was determined on three jaundiced dogs. The results are summarized in Table II.

The calcium content of the blood and tissues as enumerated above was determined on three dogs having a bile fistula. The results are summarized in Table III.

Chart 1 shows the increase and decrease of the calcium content of the tissues in jaundiced and bile fistula dogs when compared to the normal.

In jaundiced dogs there is an increase in the calcium content of the muscle, thyroid, and adrenals and a slight increase in calcium content of the bone and heart. There is a marked decrease in the serum calcium, the whole

TABLE I

DOG NO.	WT. OF DOG	SERUM	WHOLE BLOOD	DIFFUSIBLE	SPINAL FLUID	BRAIN	LIVER	SPLEEN	MUSCLE	BONE	SUBCUTANEOUS TISSUE	HEART	THYROID		LUNG	KIDNEY	ADRENAL
		Calcium per 100 c.c.				Calcium per 100 gm.											
	Kg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.
1	13.6	12.1	6.1	4.2	8.6	52.97	18.61	34.87	15.77	9,793.00	29.04	19.61	30.41	41.48	41.06	38.92	60.43
2	10.9	13.2	5.5	5.1	7.4	57.93	23.81	64.71	17.94	11,314.60	11.03	22.03	36.74	36.42	44.54	36.05	71.63
3	11.3	12.2	4.3	5.8	7.5	63.54	23.40	61.01	10.75	17,652.60	17.28	13.53	41.84	30.98	39.06	32.97	58.42
4	10.9	13.2	7.0	5.4	7.2	66.53	22.26	39.70	23.58	15,343.10	24.38	25.08	44.93	37.42	44.58	34.92	61.31
5	11.3	12.6	6.9	5.6	8.6	60.40	27.03	50.06	20.68	14,112.20	27.70	29.61	32.62	39.61	42.68	37.80	73.42
Av.	11.6	12.6	5.9	5.2	7.8	60.27	23.02	50.07	17.72	13,643.10	21.88	22.02	37.30	37.18	42.38	36.03	65.04

TABLE II

DOG NO.	NORMAL WT. OF DOG	FINAL WT. OF DOG	DURATION EXP.	NORMAL SERUM	FINAL SERUM	NORMAL WHOLE BLOOD	FINAL WHOLE BLOOD	NORMAL DIFFUSIBLE	FINAL DIFFUSIBLE	BRAIN	LIVER	SPLEEN	MUSCLE	BONE	SUBCUTANEOUS TISSUE	HEART	THYROID	GALL BLADDER	LUNG	KIDNEY	ADRENAL
				Calcium per 100 c.c.						Calcium per 100 gm.											
	Kg.	Kg.	Days	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.
6	12.5	8.8	31	11.4	8.9	6.3	4.0	4.8	1.6	35.67	26.84	26.30	28.65	16,611.01	9.11	22.98	48.32	44.71	48.57	44.33	70.84
7	15.4	12.2	40	11.5	10.4	7.0	4.0	4.6	1.3	36.40	30.10	48.08	38.57	13,722.18	10.84	29.51	40.61	50.62	48.22	40.11	74.63
8	10.9	7.4	49	12.1	8.8	4.4	3.2	4.2	.8	29.50	24.44	34.84	32.40	17,679.05	10.68	30.30	32.42	49.43	47.32	18.52	94.09
Av.	12.9	9.4	40	11.6	9.3	5.9	3.7	4.5	1.2	33.85	27.12	36.40	33.20	16,004.80	10.20	27.59	40.45	48.25	48.03	34.33	79.85

TABLE III

9	11.0	7.7	70	11.0	14.8	5.5	10.1	4.8	7.4	49.06	29.12	60.77	20.03	23,470.46	18.98	18.66	64.86	22.46	42.70	37.96	74.96
10	9.5	10.0	84	10.8	14.2	4.8	8.4	3.4	6.8	63.41	24.69	45.73	24.69	20,068.31	13.68	14.34	50.80	28.00	56.76	93.43	61.75
11	15.4	15.2	84	11.8	15.7	4.6	9.2	4.0	8.6	78.36	18.59	24.25	18.02	19,826.08	10.59	19.36	61.42	31.06	62.99	91.05	50.19
Av.	11.9	10.9	79	11.06	14.9	4.9	9.2	4.06	7.6	63 61	24.13	43.58	20.91	21,121.61	14.41	17.45	62.22	27.17	54.15	74.14	52 96

Table IV

Jaundiced Dogs

Dog No.	Days	Serum	Whole Blood	Diffusible	Dog No.	Days	Serum	Whole Blood	Diffusible	Dog No.	Days	Serum	Whole Blood	Diffusible
		Calcium per 100 c.c.					Calcium per 100 c.c.					Calcium per 100 c.c.		
6	0	11.4	6.3	4.8	7	0	11.5	7.0	4.6	8	0	12.1	4.4	4.2
6	7	9.9	5.4	2.0	7	7	11.0	4.0	1.5	8	7	11.4	4.6	4.0
6	14	11.0	4.6	3.0	7	14	11.3	3.0	1.2	8	14	11.4	5.2	4.8
6	21	11.0	4.1	3.5	7	21	11.3	4.1	1.6	8	21	10.0	3.8	1.8
6	28	9.9	4.0	1.8	7	28	10.6	4.2	1.0	8	28	12.0	3.2	2.0
6	31	8.9	4.0	1.6	7	35	11.3	4.1	1.8	8	35	10.8	4.4	1.6
					7	40	10.4	4.0	1.3	8	42	11.5	3.8	2.4
										8	49	8.8	3.2	0.8

Bile Fistula Dogs

Dog No.	Days	Serum	Whole Blood	Diffusible	Dog No.	Days	Serum	Whole Blood	Diffusible	Dog No.	Days	Serum	Whole Blood	Diffusible
		Calcium per 100 c.c.					Calcium per 100 c.c.					Calcium per 100 c.c.		
9	0	11.0	5.5	4.8	10	0	10.8	4.8	3.4	11	0	11.8	4.6	4.0
9	7	10.6	5.4	3.6	10	7	13.7	8.4	5.5	11	7	14.4	7.0	3.6
9	14	10.8	4.6	3.0	10	14	12.2	5.4	3.0	11	14	12.4	4.4	3.4
9	21	19.1	8.7	4.9	10	21	13.6	7.0	3.6	11	21	12.0	3.9	3.2
9	28	13.6	7.0	4.0	10	28	12.5	5.3	3.6	11	28	13.6	3.8	3.6
9	35	11.8	5.6	3.8	10	35	12.9	3.4	3.6	11	35	14.4	6.6	4.0
9	42	11.3	8.5	4.0	10	42	13.4	8.0	4.0	11	42	13.6	7.8	4.3
9	49	14.3	7.0	4.0	10	49	12.7	6.3	3.8	11	49	11.3	6.6	6.1
9	56	12.0	6.0	5.2	10	56	13.4	7.3	4.8	11	56	14.1	6.6	6.3
9	63	14.4	9.8	7.2	10	63	11.4	4.6	4.4	11	63	14.6	7.4	6.4
9	70	14.8	10.1	7.4	10	70	13.0	6.7	6.0	11	70	14.4	6.2	6.0
					10	77	13.8	10.5	6.7	11	77	14.0	6.8	6.0
					10	84	14.2	8.4	6.8	11	84	15.7	9.2	8.6

blood calcium, and the diffusible calcium, a decrease in the calcium content of the brain, spleen, and the subcutaneous tissue.

In the bile fistula dogs there was marked increase in the serum calcium, the whole blood calcium, and the diffusible calcium, an increase in the calcium content of the bone, thyroid, lung, and kidney. There is a decrease in the calcium content of the subcutaneous tissue, gall bladder, and adrenal and a slight decrease in the calcium content of the heart.

In each of the three jaundiced dogs and the three bile fistula dogs blood was taken each week and the serum calcium, whole blood calcium and diffusible calcium were determined. Table IV shows these results. Charts 2 and 3 show the curves of one jaundiced dog, Dog 8, and one bile fistula dog, Dog

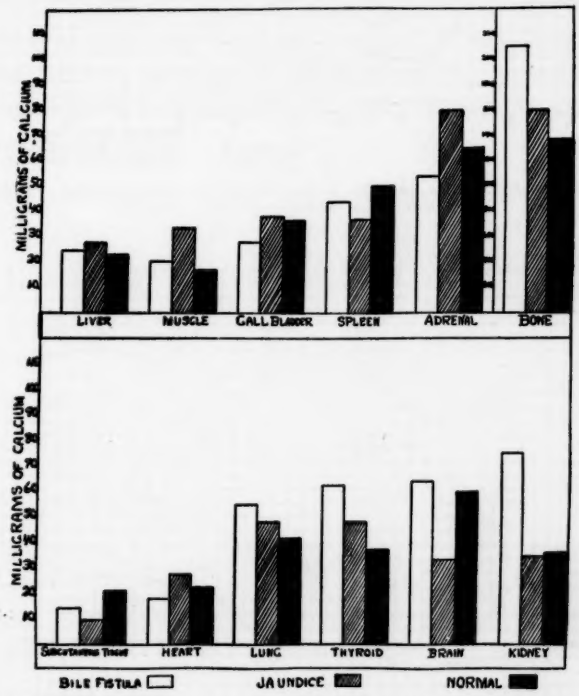

Chart 1.—The tissue calcium in bile fistula, jaundiced, and normal dogs.

11. In the jaundiced dogs there is a steady fall in serum calcium, whole blood calcium, and diffusible calcium. In the bile fistula dogs there is a steady rise in the serum calcium, whole blood calcium, and diffusible calcium.

Feces were obtained from two normal dogs, two jaundiced dogs, and two bile fistula dogs on three consecutive days and the calcium content determined. The jaundiced dogs were jaundiced twenty-eight days and bile fistula dogs had a bile fistula seventy days. These results are summarized in Table V. The calcium content of the feces of jaundiced and normal dogs is practically the same. There is a definite decrease in the calcium content of the feces of bile fistula dogs.

On one normal dog samples of blood were taken on ten consecutive days at different times of the day, the dog being on the same diet as were all the

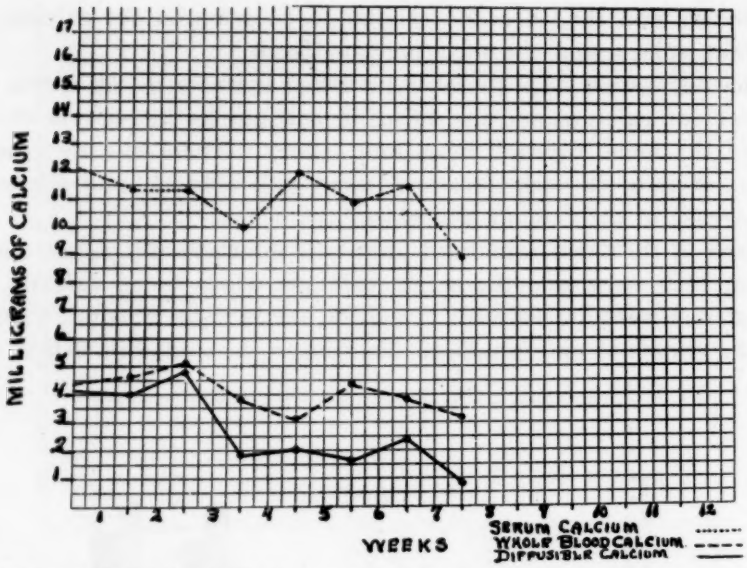

Chart 2.—Curves representing the fall in serum calcium, whole blood calcium, and diffusible calcium in a jaundiced dog. Dog 8.

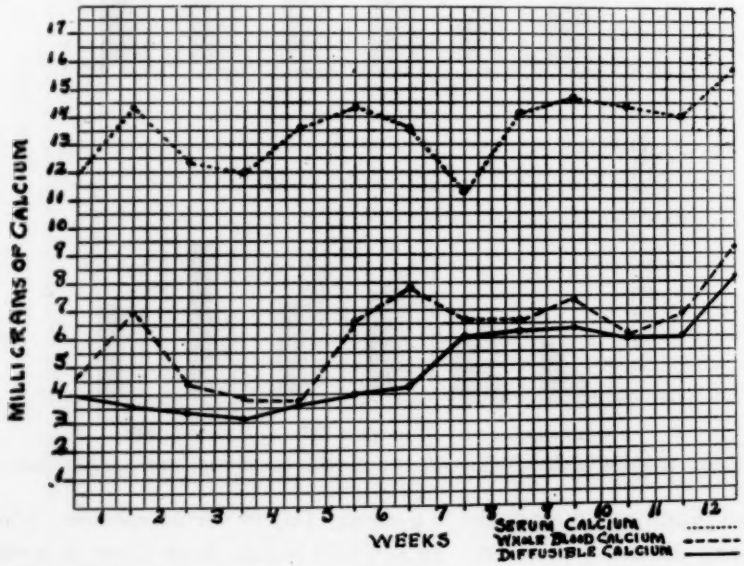

Chart 3.—Curves representing the ruse in serum calcium, whole blood calcium, and diffusible calcium in a bile fistula dog. Dog 2.

dogs used in this experiment and the serum calcium was found to vary only 0.3 of a mg.

The determination of the calcium in the bone by means of an increased or a decreased density as shown on the x-ray plate and measured by the photometer checked with the chemical determination of the bone calcium.

The calcium content of the kidney of a normal dog, the renal artery and vein of which had been clamped off, was compared with the calcium content of the opposite kidney, the renal artery and vein being intact, the dog having

been bled to death and his tissues perfused. The kidney whose artery and vein had been clamped off gave a lower calcium content than the kidney that was perfused. This indicates that perfusion does not wash out any calcium from the tissues.

DISCUSSION

The fact that in jaundiced dogs there is a very definite decrease in the serum calcium, the whole blood calcium, and especially marked decrease in the diffusible calcium would suggest that the calcium in the blood is bound by some constituent of the bile or blood. Loeb and Nichols[13] have shown that calcium is bound to certain of the blood proteins. Kirk and King[4] suggest that there may be other blood proteins that do not bind calcium and that a disturbance in the protein balance might affect the degree of diffusible calcium. The bile protein may bind the calcium of the blood also. Klinke[3] believes that the ionized calcium is adsorbed to the proteins of the blood and splits off on dialysis passing into the outer fluid. It may very will be

TABLE V

NORMAL			JAUNDICED			BILE FISTULA		
DOG NUMBER	SAMPLE NUMBER	CALCIUM IN FECES PER 100 G.M.	DOG NUMBER	SAMPLE NUMBER	CALCIUM IN FECES PER 100 GM.	DOG NUMBER	SAMPLE NUMBER	CALCIUM IN FECES PER 100 G.M.
		MG.			MG.			MG.
1	1	2972.75	7	1	1870.15	10	1	2311.55
1	2	2825.15	7	2	3276.70	10	2	1420.12
1	3	3016.15	7	3	2957.61	10	3	1418.76
Average		2934.68	Average		2701.48	Average		1716.81
2	1	3044.65	8	1	4755.44	11	1	1291.81
2	2	3388.38	8	2	2458.09	11	2	1218.17
2	3	3347.95	8	3	1775.07	11	3	551.28
Average		3260.32	Average		2996.20	Average		1020.42
Average of Both Samples		3065.00	Average of Both Samples		2848.84	Average of Both Samples		1368.62

that the adsorption of calcium by the blood proteins is decreased in jaundice. King and Stewart[5] believe that the calcium of the blood is bound by the bile pigments.

When bile is shunted out of the circulation and intestines by means of a bile fistula the increase in the serum calcium, whole blood calcium, and especially marked increase in the diffusible calcium would suggest that in this instance, there being very little bile absorbed, the calcium that was normally bound by some constituent of the bile or blood is now free in the blood stream.

There is a definite unbalance in the calcium metabolism in dogs with a bile fistula as shown by the marked decrease in the calcium of the feces and a marked increase in the bone calcium. The calcium seems to be stored in the bones.

These findings help to explain the mechanism whereby increasing the ionized or diffusible calcium of the blood by giving jaundiced patients calcium salts or parathyroid hormone will decrease the coagulation time of their blood at a time when their serum calcium is lower than normal but not nearly as low as their diffusible calcium.

CONCLUSIONS

1. There is a marked decrease in the serum calcium, the whole blood calcium and a very marked decrease in the diffusible calcium in dogs jaundiced over a period of forty days. There is also a decrease in the calcium content of the brain, spleen, and subcutaneous tissue and an increase in the calcium content of the muscle, bone, adrenal, heart, and thyroid.

2. In dogs with a bile fistula over a period of seventy-nine days, there is a marked increase in the serum calcium, whole blood calcium, and a very marked increase in the diffusible calcium. There is also a marked increase in the calcium content of the bone, an increase in the calcium content of the thyroid, lung and kidney, and a decrease in the calcium content of the feces, subcutaneous tissue, heart, gall bladder, and adrenal.

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THE RÔLE OF COMPLEMENT IN HEALTH AND DISEASE*

A Clinical Study of the Hemolytic Complement of Human Sera

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THE scientific study of serology dates from the year 1886, when Nuttall[1] and Buchner[2] made their first observations on the rôle played by a substance that was inimical to living bacteria found in the majority of fresh sera. Because of the peculiar protective property displayed by this substance, it was called "Alexin."

This important observation split the newly formed school of immunology into two camps. The followers of Metchnikoff[3, 4] upheld the cellular theory, while Flugge, Nuttall, Buchner, Pfeiffer,[5] and von Behring[6] were the standard bearers of the humoral school. The major part of our early knowledge concerning this protective substance we owe to the spirited controversy between these two schools, and as a result the fundamental principles of the science of serology were finally laid down by Ehrlich[7] and Bordet.[8]

Unfortunately, the study of this protective substance entered the field of academic discussion rather than practical application, and after the exposition of its nonspecificity in serologic phenomena,[9, 10] its importance as an "Alexin" was gradually lost sight of. Subsequent literature offers only fragmentary or indirect evidence of its rôle as a protective mechanism, conceived by Buchner.

The significance of alexin has been most forcibly called to our attention in the work with immune sera and therapeutic and protective vaccines. In the treatment of the chronic infective arthritides by vaccines, patients with a high alexic (complementary) titer nearly always gave a favorable response, but with a low or negative one the result of treatment was doubtful or unfavorable. We also observed that in long standing debilitating diseases, the complementary titer was usually very low, and in cases nearing the fatal outcome of subacute or chronic septicemia the complement was markedly subnormal if not entirely absent.

The persistent recurrence of these findings led us to believe that a careful classification of the variations of alexic titer might prove of useful prognostic value and so we undertook the present study.

Before entering into the discussion of this research, we will first define the term "complement." Alexin (Buchner), complement (Ehrlich), or addiment (Bordet) is that particular proteolytic property of active sera which reacts only in the presence of a specific antibody, a bacteriolysin, cytolysin, or proteolysin. This definition is open to criticism, owing to the protean nature of the complement, but the majority of investigators of the subject are in agreement that it is a nonspecific serologic entity.

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TECHNIC OF THE TITRATION OF THE COMPLEMENTARY VALUE

Our titration of complement is based on the hemolytic value against sheep cells which we take as an index to the bacteriolytic and proteolytic properties of the complement as well. The individual human sera are titrated in amounts varying from 0.01 to 0.10 c.c. The total volume is made up to 0.50 c.c. by the addition of physiologic saline. After an incubation of ten minutes, 0.50 c.c. of a 2 per cent sheep cell suspension is added to each tube. The cell suspension is already sensitized with two units of antisheep hemolysin. On further incubation for fifteen to thirty minutes, the least amount of serum capable of completely laking the added cells is taken as the complementary (hemolytic) titer.*

Our studies of complement can be divided into two main groups: First, the determination of the average combined hemolytic titer in the normal individual and under normal circumstances, in contrast to its variations in some of the common infectious diseases. Second, research bearing on the origin and nature of complement.

THE AVERAGE HEMOLYTIC TITER OF HUMAN SERA

In a series of two thousand apparently normal cases the average hemolytic complementary titer was found to be represented by 0.04 c.c. of active serum.

THE HEMOLYTIC TITERS IN ACUTE INFECTIOUS DISEASES

The following is a list of infectious diseases investigated: typhoid fever, lobar pneumonia, acute rheumatic fever, erysipelas, osteomyelitis, acute miliary tuberculosis, bacterial endocarditis, and liver abscess. The appended tables are selected from many as group representatives.

Group 1.—Acute infectious diseases running a normal course and ending in recovery without complications.

In this group we have selected one case each of four different but typical acute infections: lobar pneumonia (Fig. 1), erysipelas (Fig. 2), acute rheumatic fever (Fig. 3), and typhoid fever (Fig. 4).

The complementary curves in all cases are practically identical and may be summed up as follows:

With the onset of an acute infection, the stage at which patients are usually admitted to the hospital, there is an increase in the complementary titer. With the progress of the disease and during the fastigium, this titer stays above normal with only slight fluctuations. With the fall of the temperature, whether by crisis or lysis, the complementary titer also falls, and even though the patient be free of fever the complementary titer remains at subnormal value for about a week, at the end of which time it completes the negative phase of its cycle and mounts gradually in daily steps to reach normal value again in another week.

*As the majority of human sera naturally contain some antisheep hemolysin, the titer as determined above is the combined hemolytic and not the pure complementary value. The effect of this native hemolysin, in the amounts in which it normally occurs, is of minor significance since titrations carried on with it and those performed without it by the absorption method showed only slight differences.

Group 2.—Cases similar to Group 1, except for a pseudo-fall of temperature, or pseudocrisis.

We present two cases as representative of this group. One, a lobar pneumonia, with pseudocrisis (Fig. 5); the other, a typhoid fever with a third week pseudo-variation in temperature (Fig. 6).

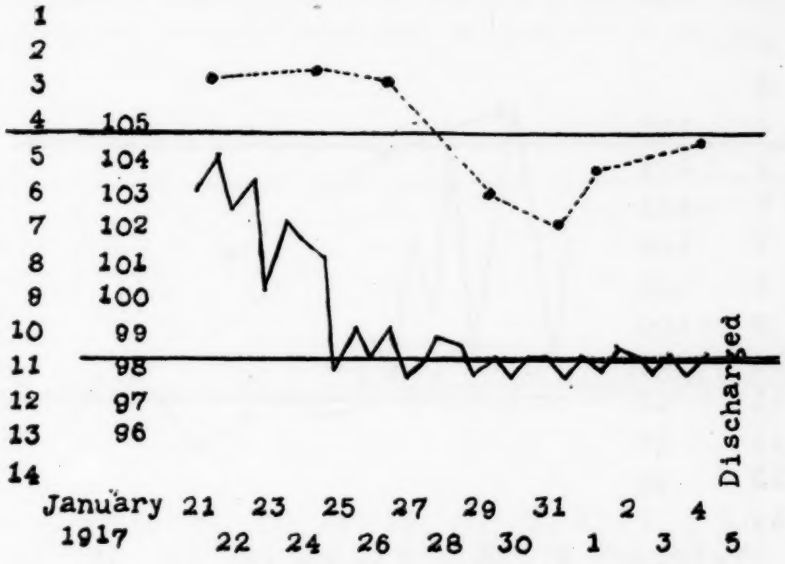

Fig. 1.—Case J. R. Pneumonia.

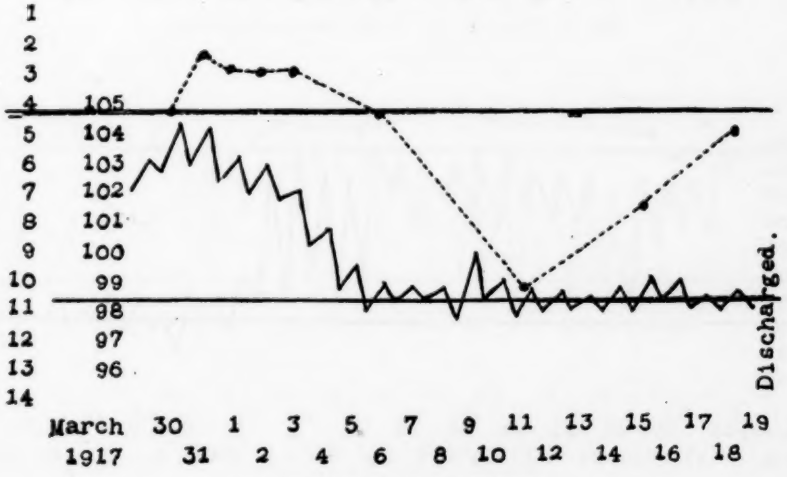

Fig. 2.—Case K. F. Erysipelas.

Without going into the theory of the nature and production of complement, which we discuss in the latter part of this paper, we venture to draw the following conclusions in order to make the study of the group under discussion easier of comprehension. The close parallelism between fever and complement as represented in the first group tempts us to consider them together and as influenced by the same cause. Whatever may be the immunologic changes during a true crisis, the effect on temperature and complement

is almost identical. In a pseudocrisis, however, the complement, unlike the temperature, departs from its normal course and with the secondary rise of temperature continues to fall until it becomes nearly twice as low as in a true crisis. But, in the absence of true complications, it gradually goes back to its normal course and eventually reaches its normal level.

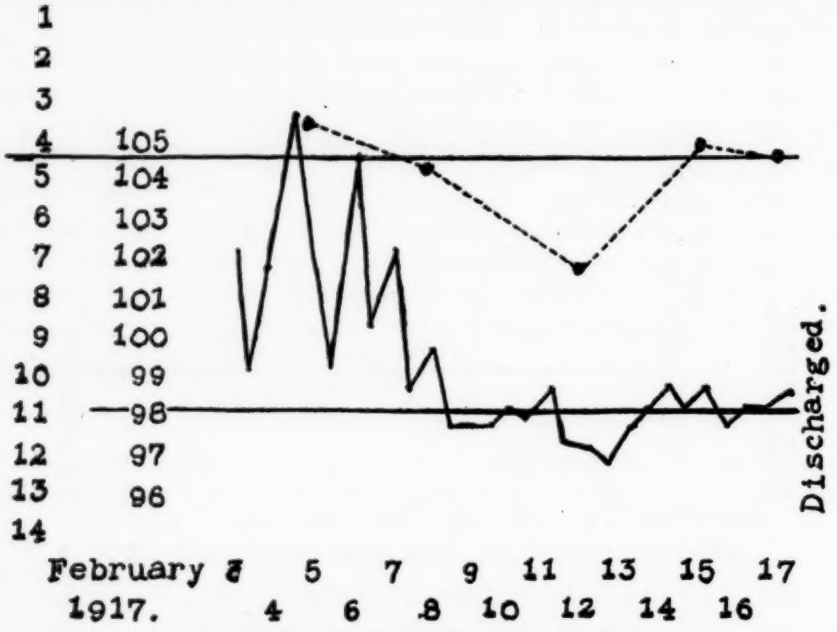

Fig. 3.—Case M. P. Acute rheumatic fever.

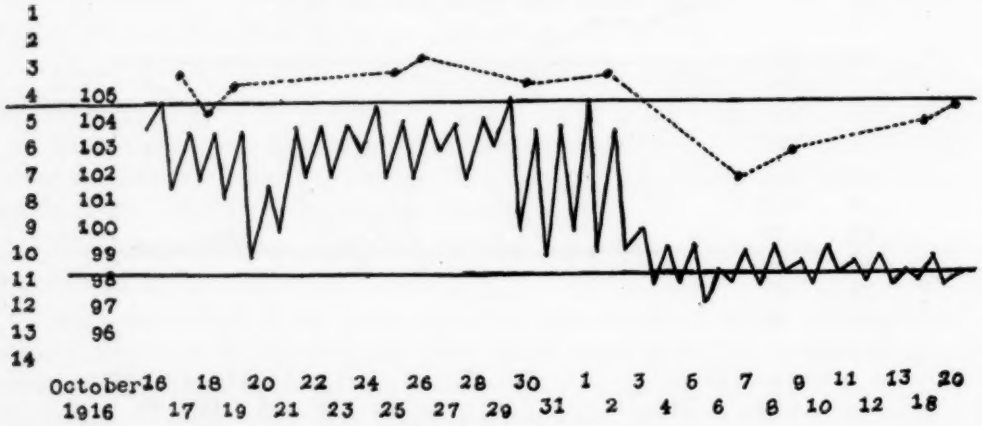

Fig. 4.—Case C. Th. Typhoid Fever.

Group 3.—Cases with relapse and complications.

Two cases of erysipelas, one with a relapse (Fig. 8), the other with relapse and complication (Fig. 7), and a case of lobar pneumonia (Fig. 9) are included in this group.

The cases given under this group are clinically different from Group 2, and their complementary curves deviate considerably in the following points from the course taken in the preceding group:

If the relapse occurs during the fall of complement, there is a still further fall in the titer of the latter similar to that in pseudocrisis reactions (Fig. 7). If the relapse occurs following the completion of the negative phase of the complement value, then there is a sharp ascent, the subsequent course being identical with the acute infections cited under Group 1 (Fig. 8). If the relapse is a severe one and its course unduly prolonged and coupled with a steady fall of complementary titer, the prognosis is very grave (Fig.

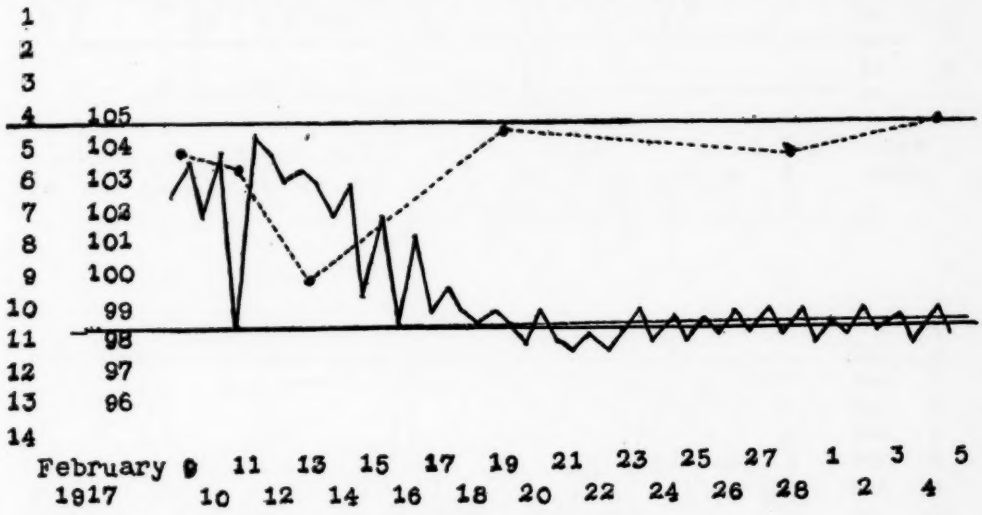

Fig. 5.—Case J. P. Pneumonia.

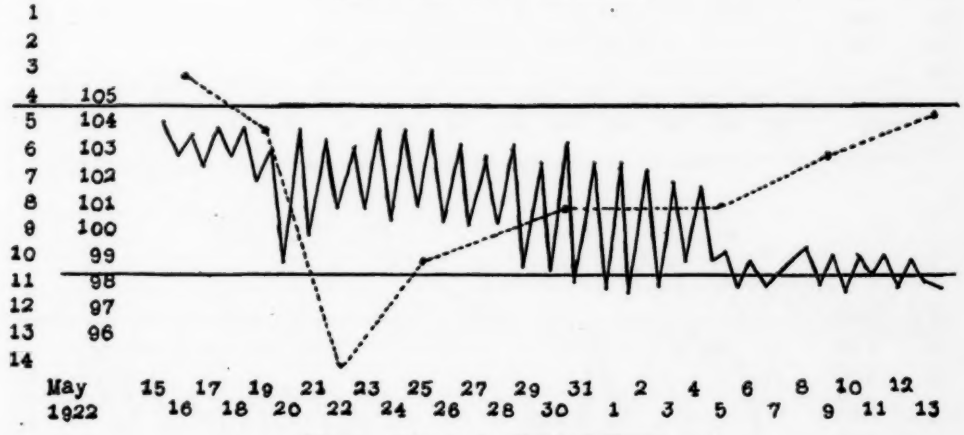

Fig. 6.—Case I. C. Typhoid Fever.

9). This case could not be followed to the end, but when he was removed from the hospital by his relatives he was extremely toxic.

Group 4.—Cases of acute and chronic infections terminating fatally.

Three cases have been chosen to represent this group; one, a case of erysipelas (Fig. 10), one of chronic rheumatic endocarditis (Fig. 11), and one of chronic pulmonary tuberculosis (Fig. 12).

In all of these cases the complementary titers were persistently subnormal from the time they entered the hospital. The daily fluctuations were

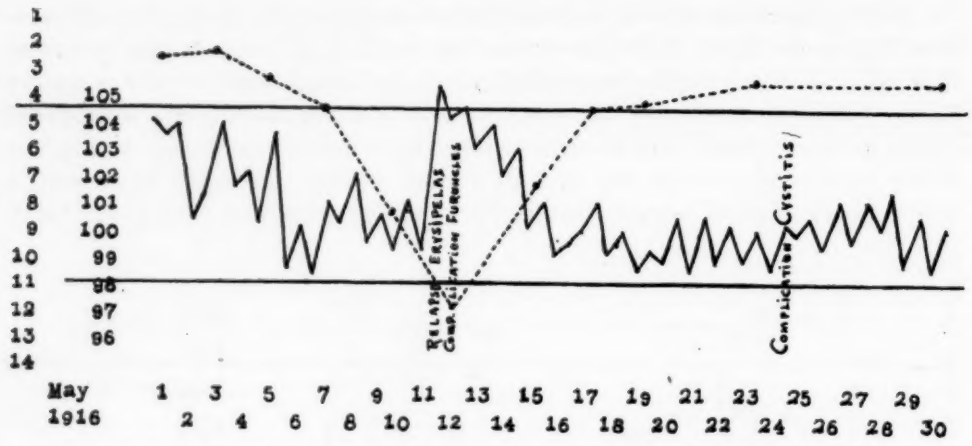

Fig. 7.—Case Th. K. Erysipelas with relapse and complicated by cystitis.

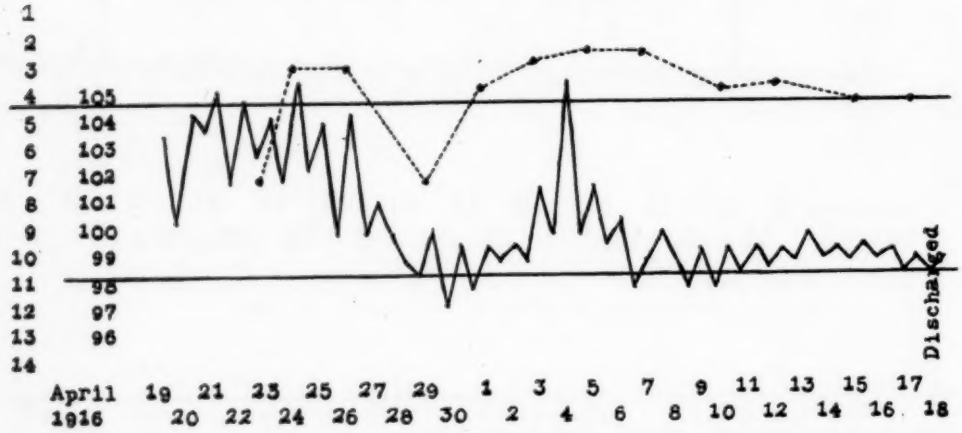

Fig. 8.—Case J. D. Erysipelas with relapse.

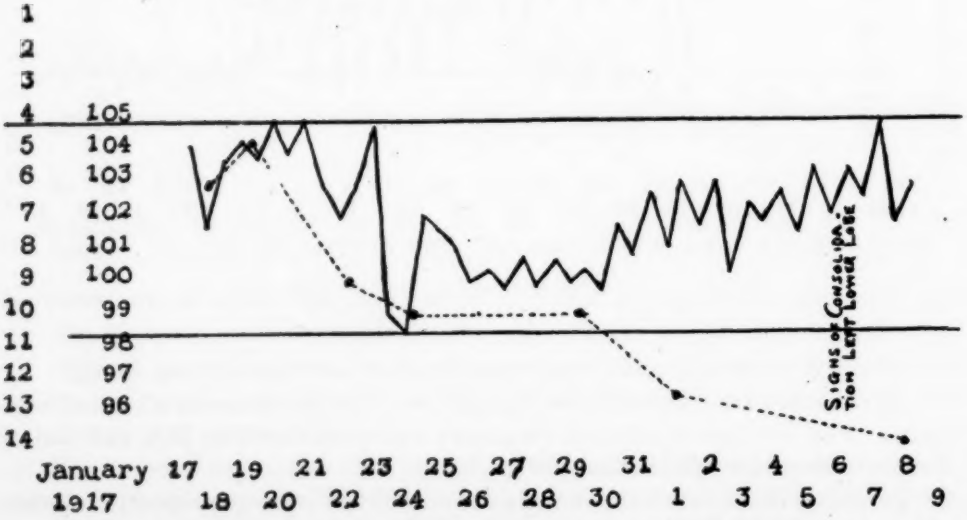

Fig. 9.—Case J. M. Lobar pneumonia with recurrence in the opposite lobe.

slight, either for better or worse, until there was a fall well below ordinary subnormal limits with no tendency toward improvement. In the case of B. B. (Fig. 10) a fatal prognosis was made almost a week before it actually occurred, because of the sudden fall in complementary value at that time. Case S. D. (Fig. 11), suffering from chronic rheumatic endocarditis, was dis-

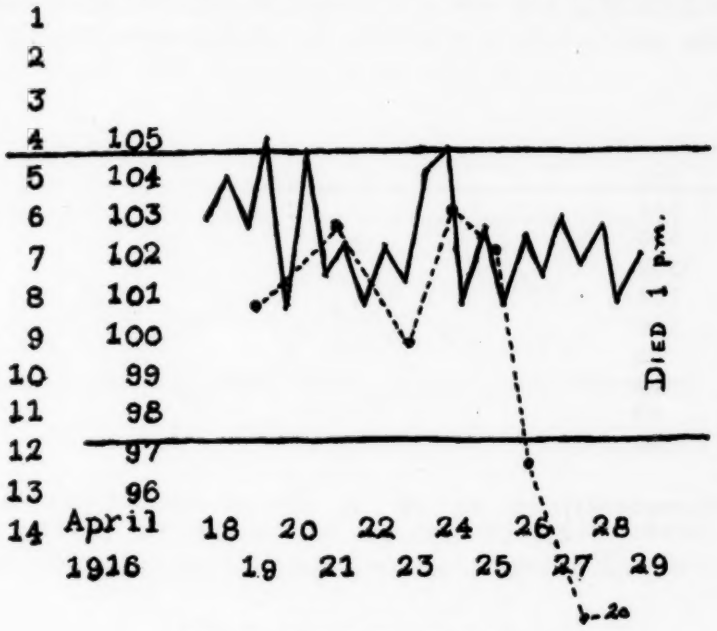

Fig. 10.—Case B. B. Erysipelas.

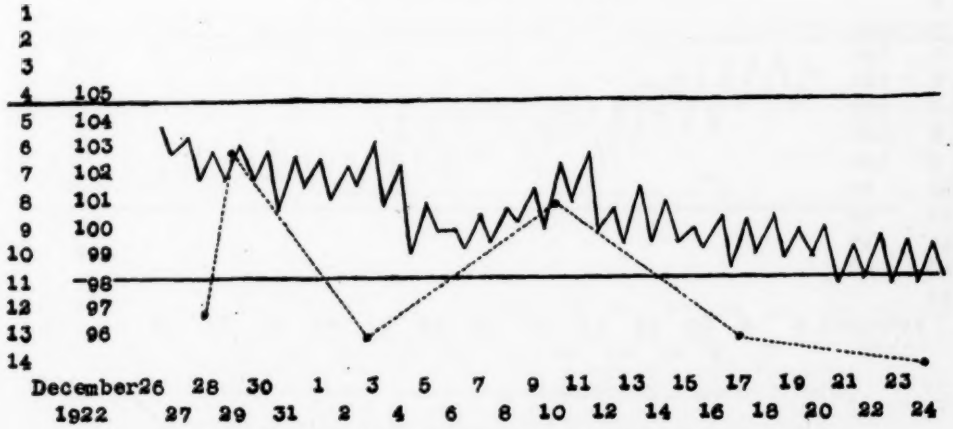

Fig. 11.—Case S. D. Acute rheumatic endocarditis (bacterial).

charged from the hospital apparently holding his own. During his stay in the hospital the complement curve was persistently subnormal. In spite of repeatedly negative blood cultures, the prognosis on discharge, based on the complement, was unfavorable. Two months later he was readmitted with heart badly decompensated. At this time the blood culture was positive for Streptococcus viridans. He died shortly after the second admission. Case

A. B. (Fig. 12), in view of the preceding discussion, needs little comment. Clinically, the patient was badly run down and had disseminated tubercular involvement of both lungs, so that the prognosis even without the complement curve was evident.

Group 5.—In this group we include three cases of grave septicemia, of which Case I. B. (Fig. 13), one of osteomyelitis with Staphylococcus aureus blood culture, and Case D. P. (Fig. 15), one of deep-seated inguinal abscess

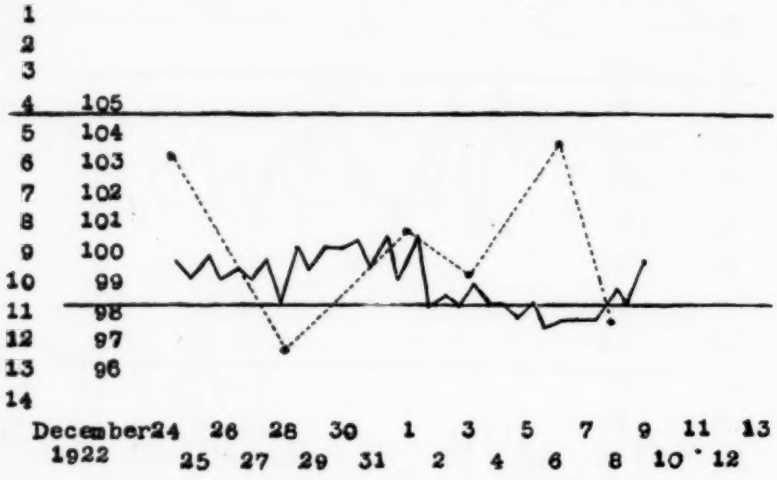

Fig. 12.—Case A. B. Chronic pulmonary tuberculosis.

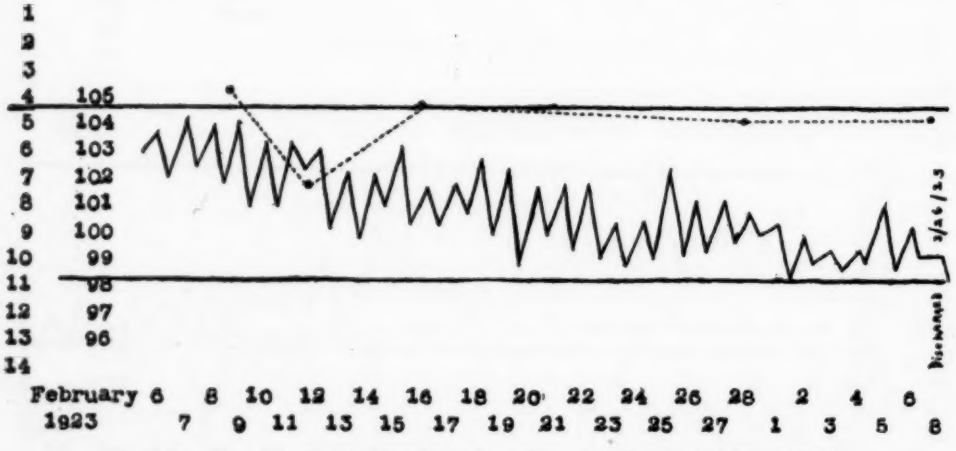

Fig. 13.—Case I. B. Staphylococcus aureus septicemia (osteomyelitis).

with Streptococcus hemolytic blood culture, both had long septic courses, but finally recovered and were discharged with sterile blood cultures, apparently completely cured. Case G. P. (Fig. 14) a subacute bacterial endocarditis, with persistently positive blood culture (Streptococcus viridans), was discharged three months after admission, on his own insistence to go home, with a fever varying between 99° and 100° during the latter part of his stay in the hospital. The subsequent course of the disease in this case unfortunately could not be followed. In view, however, of the gravity of the nature of

infection, with a complementary titer holding its own, we presume that he had an abnormally long duration for a known fatal condition.

In general, even in this group of systemic infections, we find the complement undergoing the same phases as met in infectious conditions of lesser severity. The first effect of infection on the complement, after a slight fluctuation in the latter's titer, is the negative phase (the serologic shock period). The subsequent course of the disease depends on the ability of the complement to cope with the extraordinary demand.

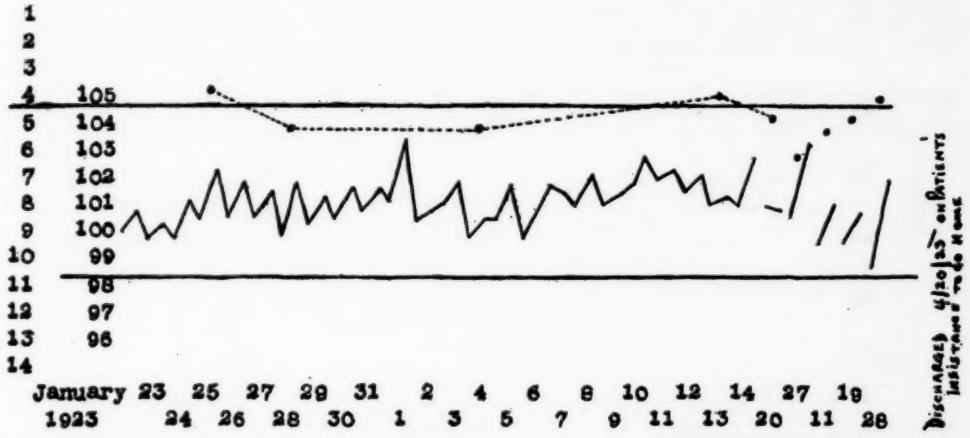

Fig. 14.—Case G. P. Streptococcus viridans septicemia. (Subacute bacterial endocarditis.)

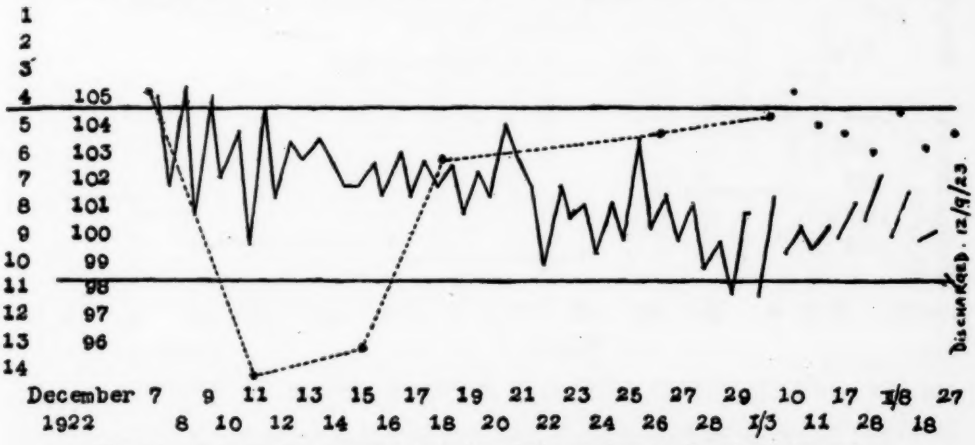

Fig. 15.—Case D. P. Streptococcus hemolytic septicemia (inguinal abscess).

RESEARCH BEARING ON THE ORIGIN AND NATURE OF COMPLEMENT

The question of the origin and nature of complement for the most part lies in the realm of pure speculation. The exhaustive studies of Ferrata[11] and Brand[12] disclosed its composite protein structure (albumin and globulin). It was further fractioned by Michaelis and Skwirsky[13] into a midpiece representing the globulin fraction and an end-piece, the albumin fraction.

Its origin has supposedly been traced to various internal organs: to the thyroid gland by L. Fassin,[14] the liver by Nolf.[15] Serologically it is con-

sidered as a nonspecific immune element.[16] Yet, Ehrlich and his school[17, 18] feel that there is a relative specificity in the bactericidal, the hemolytic, and the cytolytic complements.

We do not pretend to feel that we have solved the mystery of complement, and if we venture to give certain conclusions based on our studies, we do so merely in an attempt to throw light on a phase that has hitherto been neglected. We wonder if the complement is not a *catabolic product* which the living organism makes an economic use of in preventing and actually fighting disease. If it be catabolite, then the complementary titer is determined by the balance of production over destruction (elimination or absorption). Complement as such is not eliminated through the regular channels

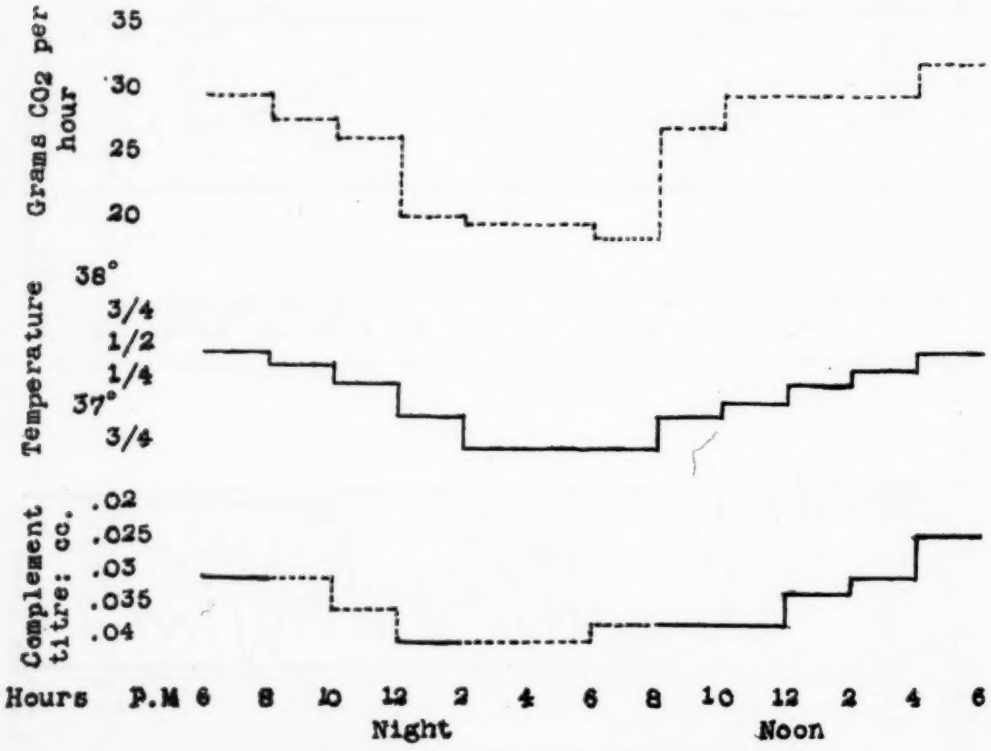

Fig. 16.—Diurnal variations of metabolism, heat, and complement.

in urine, feces, or perspiration, consequently there must be an absorption or destruction in the physiochemical changes within the human system.

Known pathologic conditions that give rise to high metabolism, such as hyperthyroidism, were also found by us to give a high complementary titer, while the reverse was found true in true hypothyroidism, cretinism, and myxedema. In acute infectious diseases, fever, taken as an index to metabolic rate, can also be used as an index to the complement. When there are departures from the above findings, such as the negative complementary phase in the early course of infections, they can be explained on the basis of an excessive consumption rather than on that of deficient production. We are already familiar with the complement neutralizing effect of protein cleavage

products, such as the peptones and albumoses, and it would seem reasonable to suppose that the consumption or inactivation of complement could be ascribed to the direct effect of proteolytic by-products derived from bacteriolysis or nonspecific proteolysis under high fever.[19, 20, 21]

We have collected certain experimental data in support of the catabolic theory which will be cited briefly.

Diurnal Variations of the Complement.—Fig. 16 gives the daily variations of the complement in relation to similar variations of body temperature and metabolism. The complement curve is the average of three findings under normal conditions. The temperature and CO_2 elimination curves are quoted from Lusk, *Science of Metabolism,* third edition.

Effect of Exercise on Complement.—Fig. 17 gives the initial complementary titer before exercise and then immediately following, and five minutes

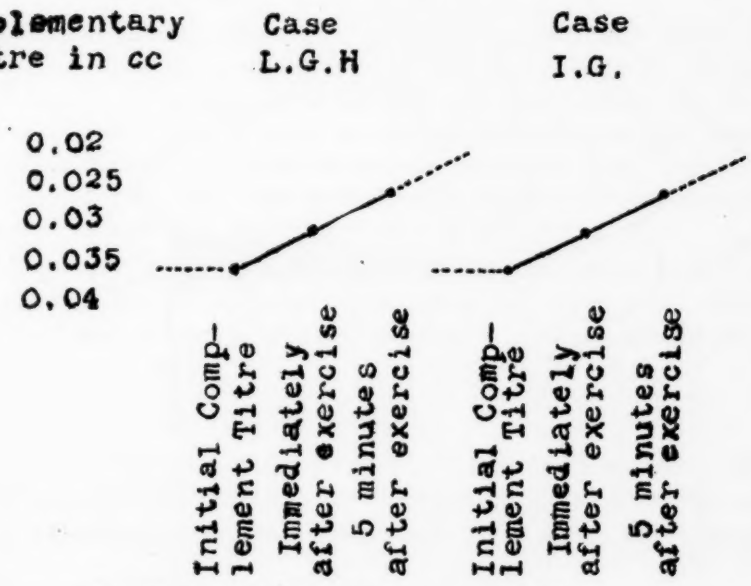

Fig. 17.—The effect of exercise on complement.

after the strenuous exercise of climbing seventy-two steps three times. The distinct rise of the complement titer is evident. Unfortunately we did not extend our observations for longer periods to determine the duration of the hypercomplementary stage for comparison, as calorimetric studies of exercise usually show a lagging of the high caloric output.

The Complementary and Metabolic Cycles in Double Tertian Malaria.—In the previous observations in acute infectious diseases, no attempt was made to determine the hourly fluctuations of complement in relation to similar fluctuations in fever. In view of the fact that it is almost impossible to secure cases before the onset of temperature, such an attempt would not have answered the question we had in mind and our choice appeared to be limited either to producing a febrile reaction artificially by means of protein injections or to watching the course of a true relapse. Fortunately at this time a

case of double tertian malaria was admitted to the hospital from the dispensary service and was an ideal object for study. Two full cycles were studied and the results given in Fig. 18.

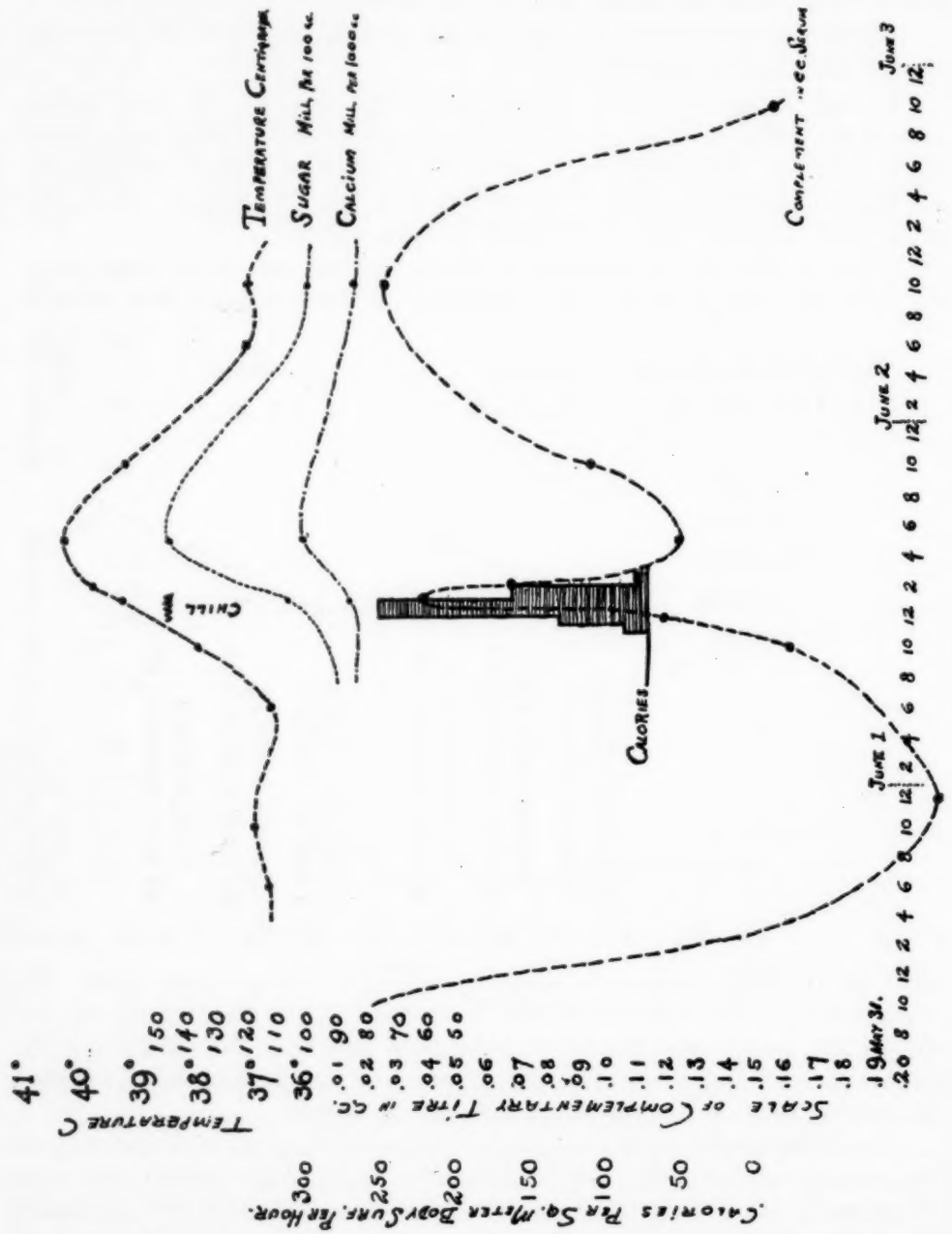

Fig. 18.—Hourly complementary and metabolic variations in a malarial paroxysm.

As we did not have the facilities for calorimetric determinations of the metabolism during the same period we have inserted those by Barr and DuBois,[22] taking the period of chill for our landmark.

In interpreting the complementary curve it is necessary to take into consideration, first, that with a double tertian malaria there is only one day of afebrile period and, second, that the values obtained are mean values, namely, the balance of complement production over destruction. The termination of a febrile course, such as found in malaria, gives a complement steadily falling to subnormal values, in all probability because of deficient production rather than excessive destruction. If such be the case, it would point to exhaustion of the complement producing sources and if our feeling that complement is a catabolite be correct, it would mean a relative reduction in the afebrile period, a supposition which we discovered to be true.

With the onset of the paroxysm and as we enter the chill period, there is a sharp rise in complement as well as metabolism. The metabolic rate reaching its peak about an hour earlier than the complement would point strongly to the catabolic nature of the latter.

Immediately following the chill, while the temperature is still mounting to its fastigium, both the complement and the metabolic rate fall sharply. The fall of the latter becomes more gradual when basal values are reached. In the case of complement, however, the fall exceeds the regularly subnormal values until the fastigium in fever is reached, at which stage there occurs a gradual upturn in the curve and with the now steadily falling temperature reaches normal values, thus bringing us to the end of the cycle.

The fall in complement immediately following the chill we ascribe not to failing production but to an increased reduction or inactivation caused by the sudden explosive discharge of proteolytic split products during the stage of chill.

CONCLUSIONS

1. During an infectious disease the course of the complementary, "alexic" value of the patient's serum shows considerable variation from the normal.

2. Such variations have been demonstrated by us to have prognostic significance.

3. With the onset of disease there is a steady *rise in the production of the complement.*

4. This stage passes rather abruptly into the *negative alexic phase,* the serologic shock period, with an abnormal fall in the complement. At this stage the fall of the alexic titer is due, in all probability, to increased complement destruction or absorption from the sudden flooding of the blood stream with proteolytic by-products of known anticomplementary properties.

5. With the establishment of recovery and during convalescence the ratio between complement production and destruction is reversed, and in the course of a week the titer gradually mounts to normal values and stays there.

6. Deviation from this regular course occurs in oncoming complications, relapse or recrudescence. But a gradually mounting complement is a favorable prognostic sign. In fatal cases, the titer either falls to very low values or runs a relatively subnormal course.

7. The close relationship between the (calorimetric) metabolic and complementary studies in acute infectious diseases compels us to consider the complement as a catabolic product of which the animal system makes an economic use.

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EXPERIMENTAL ACUTE MERCURIALISM*

By Samuel Goldblatt, M.D., M.S., Cincinnati, Ohio

THE following experimental work was carried out in the attempt to throw some light upon the conflicting phenomena described in acute mercurialism.

The blood chemistry of normal dogs was determined and compared with the findings in similar animals which had received lethal and sublethal doses of mercuric chloride intravenously and by mouth under various conditions.

METHOD

The animals were kept in metabolism cages and fasted for twenty-four hours before the experiment. All injections were made into and the blood withdrawn from the external jugular vein. Water, and food consisting of bread, were allowed ad libitum during the course of the experiment. Blood was obtained every twenty-four hours for chemical analysis, unless otherwise stated on the accompanying protocols.

The urea nitrogen was determined by the Van Slyke and Cullen[1] modification of the Marshall method. A protein-free filtrate was obtained by the Haden[2] modification of the Folin-Wu technic. From this filtrate the blood chloride content was determined as sodium chloride after the manner described by Gettler,[3] the sugar by the method of Folin and Wu.[4]

Animals in which the effect of intravenous bichloride was observed received various amounts of a standard solution containing 4 grams $HgCl_2$ to a liter of distilled water. This was injected slowly into the external jugular vein.

The influence of cholecystostomy was determined in a small group of animals. They were fasted for twenty-four hours, anesthetized with ether, and operated upon under aseptic technic. The common duct was exposed, ligated, and resectioned between the ligatures. A tube was inserted into the gall bladder or the gall bladder was sewed to the abdominal wall making a permanent biliary fistula. When the animal recovered from the effect of the operation it received mercuric chloride intravenously. The chemistry of the blood was studied daily.

The time required for the absorption of a lethal dose of the poison was determined upon a series of 75 dogs. This was carried out in the following manner:

Thirty-three animals were given mercuric chloride as the dry powder or in aqueous solution. The time of first emesis was noted and controlled. Ten dogs of this group were selected for a study of the blood chemical changes following the various emesis intervals.

*Received for publication, April 24, 1928.

TABLE I

PROTOCOL OF EXPERIMENTS. BLOOD CHEMISTRY. AMOUNT OF BLOOD PER 100 C.C.

DOG NO.	WT. KG.	TIME DAYS	UREA N. MG.	CHLORIDES NaCl MG.	CO_2 VOL.	GLUCOSE MG.	N.P.N. MG.	REMARKS
1	6	1	12	480	43	100	28	
		2	10	480	37	87	21	
		3	7	440	35	85	31	
		4	9	480	37	83	27	
		5	10	520	38	90	28	
		6	11	530	35	82	34	
		7	8	540	41	75	32	
2	13	1	10	430	40			26 mg. $HgCl_2$ intrav.
			14	410	37			Reading sixth hour
		2	66	410	34			
		3	63	400	35			
		4	46	390	36			Dog looks well
		5	35	340	38			Dog looks well
		6	21	400	40			Dog looks well
		7	17	350	49			Dog looks well
		8	20	390	41			Dog looks well
		9	14	420	42			Dog looks well
		10	14	430	42			Dog looks well
		30	16	440	43			Had shown no symptoms or changes in blood chemistry since tenth day
		60	14	460	45			
3	15	1	10	500	45	80	26	14 mg. $HgCl_2$ intrav.
		2	31	420	35	64	49	
		3	94	350	21	64	137	Plasma bile stained
								Dog very sick
		4	112	250	24	71	183	Plasma bile stained
								Dog very sick
		5						Dog died during night
4	18	1	8	550	39			60 mg. $HgCl_2$ intrav.
		2	28	530	44			
		3	56	490	41			
		4	54	450	37			
		5	55	400	35			
		6	56	400	40	71		Refuses food
		7	60	430	37	79		
		8	68	430	34	71		Dog looks sick
		9	80	430	34	67		
		10	91	400	39	83		Appetite returns
		11	102	450	37	96		
		12	111	470	34	128		Looks very sick
		14	139	500	31	120		
		15						Dog died
5	10	1	24	450	40			40 mg. $HgCl_2$ intrav.
			21	460	36			Reading fourth hour
		2	56	380	25			
			59	410	23			Reading fourth hour
		3	110	400	17			
			125	400	22			Reading fourth hour
		4						Dog died
6	10	1	21	460	41			60 mg. $HgCl_2$ intrav.
			14	420	43			Reading fourth hour
			35	400	30			Reading eighth hour
			45	400	28			Reading thirteenth hour

TABLE I—CONT'D

DOG NO.	WT. KG.	TIME DAYS	UREA N. MG.	CHLORIDES Nacl MG.	CO_2 VOL.	GLUCOSE MG.	N.P.N. MG.	REMARKS
			52	400	25			Reading twenty-third hour
			91	380	20			Reading thirtieth hour
								Dog died thirty-sixth hour
9	14	1	21	450	40			84 mg. $HgCl_2$ intrav.
			28	440	36			Reading fourth hour
			42	420	21			Reading eighth hour
			38	450	10			Reading thirteenth hour
			52	400	10			Reading twenty-third hour
		2						Dog died thirtieth hour
10	14	1	15	450				112 mg. $HgCl_2$ intrav.
			24	650				Reading first hour
			28	450				Reading sixth hour
		2	76	420				
		3	154	370				
		4	138	350				
		5	196	290				
		6						Dog died

A series of 14 animals received oral lethal doses of the bichloride dissolved in 500 to 750 c.c. of milk.

In a third group experiments with calcium sulphide as an immediate antidote were carried out. One grain tablets of calcium sulphide were utilized and were found to be extremely difficult of solution, even in boiling water. Aqueous solution of 7.5 grains of bichloride was administered to the animal by stomach tube and was followed immediately by a solution of 8 grains of calcium sulphide. This was carried out in 10 dogs.

In another group of 8 dogs the bichloride was dissolved in milk, administered by stomach tube, and followed immediately by a solution of 8 grains of calcium sulphide.

In the last group of 10 dogs aqueous solution of mercuric chloride in lethal oral doses was administered. Gastric lavage was performed at definite intervals with a solution of 1 gram of sodium thiosulphate. This was immediately followed by a solution of 1 gram of thiosulphate which was permitted to remain in the stomach.

EXPERIMENTAL OBSERVATIONS

In a series of five dogs, the normal values for the blood chemistry were determined. These values are approximately the same as those described by other authors.

Chemical changes in the blood after the intravenous injection of mercuric chloride are shown in a series of twenty animals.

Four dogs received 2 mg. per kg., of which three died and one recovered. The blood chemical findings in the recovered case and in one of the animals which failed to recover are shown in Chart 1.

Changes after 3 mg. per kg. were determined on three dogs. All the animals succumbed, having shown the characteristic increase in urea and nonprotein nitrogen and decrease in the chlorides, glucose and alkali reserve.

Six animals were given an injection of 4 mg. per kg. Death supervened within a variable time. The typical changes in the blood chemistry, symptomatology and at necropsy were demonstrated.

Four animals received 6 mg. per kg. intravenously. The only way in which these animals differed from the preceding group was in the increased rapidity of the changes.

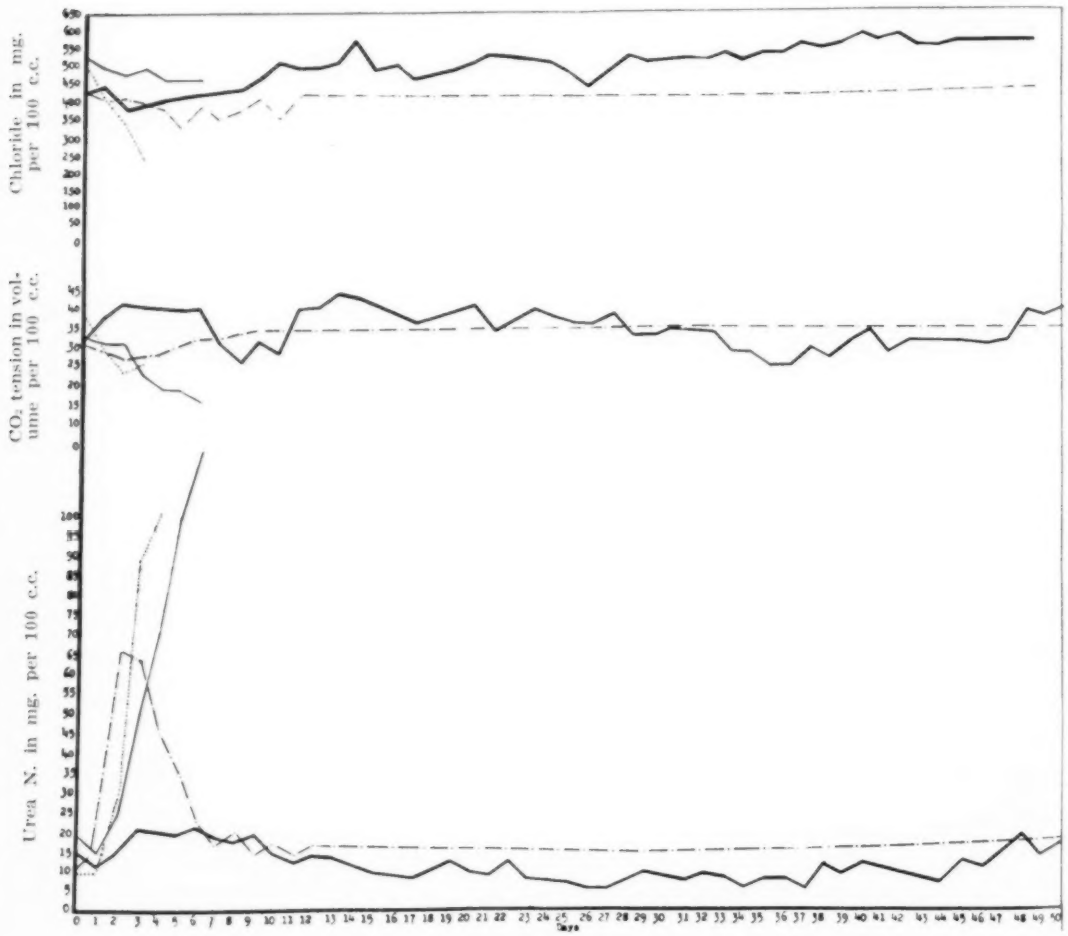

Chart 1.—Blood chemistry of $HgCl_2$ intravenously, 2 mg. per kilogram.
Normal control animal, recovery —.—.—.—
Normal control animal, fatal .....
Gall bladder drainage animal ————
Gall bladder drainage animal with hepatic disease ————

The last series consisted of three animals which received 8 mg. per kg. Owing to the rapidity of death produced by these doses when administered intravenously, as shown in Chart 2, it was necessary to resort to the intravenous administration of sodium thiosulphate in order to keep the animals alive for a study of the blood chemistry. The changes noted under these conditions were the same as in the 4 mg. group.

In Table II are shown the results of the intravenous injection of mercuric chloride in 2 to 3 mg. doses per kg. in five animals who have had a previous cholecystostomy. The complete inhibition of symptoms and of the characteristic changes in the blood chemistry even on repeated injections of the drug is significant. However, owing to the fact that only a small series of animals were utilized, although they were carried over long periods of time, definite conclusions as to the value of this procedure as a treatment for human acute mercurialism must be held in abeyance pending further research.

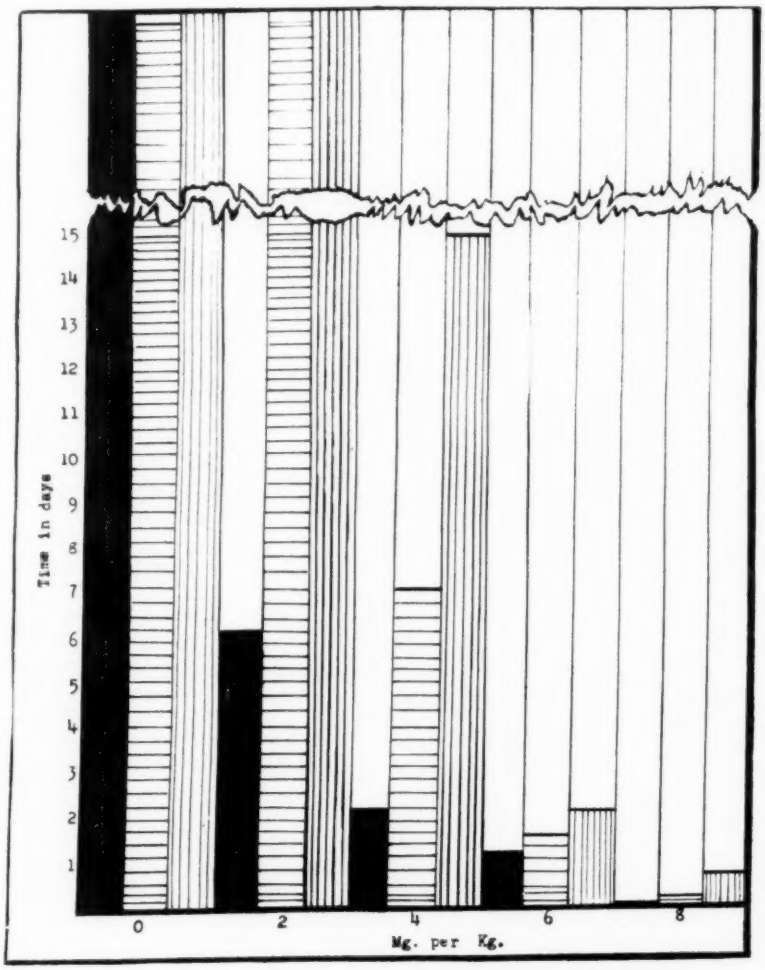

Chart 2.—Duration of life under various doses. Intravenous administration.
Minimum ▬ Mean ═══ Maximum ▒▒▒▒

A consideration of Table III shows that dogs might die after having retained the poison for eleven minutes, but did not usually die unless the drug had been retained for at least thirteen minutes. It was found that the symptoms of mercuric chloride poisoning might occur after any emesis interval (duration of time in minutes from the ingestion of the poison to first vomiting), but are not pronounced for more than one or two days unless the

TABLE II

CHEMICAL CHANGES IN BLOOD AFTER INTRAVENOUS INJECTION OF MERCURIC CHLORIDE. EFFECT OF CHOLECYSTOSTOMY

NO. DOG	KG. WT.	DAYS TIME	N. UREA MG.	CHLORIDES Nacl MG.	CO_2 VOL.	GLUCOSE MG.	N.P.N. MG.	REMARKS
3	12	1	14	440	30	45	45	
		2	10	450	36		25	24 mg. intravenously
		3	15	390	40		36	Looks well
		4	20	400	39		49	Looks well
		5	20	420	38		50	Looks well
		6	19	410	38		37	Looks well
		7	20	420	38		44	Looks well
		8	19	420	29		38	Looks well
		9	16	420	24		46	Looks well
		10	18	440	29		36	Looks well
		11	15	470	26		35	Looks well
		12	12	520	38		34	Looks well
		13	14	500	38		28	Looks well
		14	13	500	42		28	Looks well
		15	11	520	41	98	32	Looks well
		16	9	590	37	76	30	Looks well
		20	8	500	34	70	28	Looks well
		21						Drainage tube out
		26	11	510	37	71	39	24 mg. intravenously
		27	10	470	39	63	35	Looks well
		28	9	490	32	76	31	Looks well
		31	12	500	38	58	23	Looks well
		32	8	520	35	67	18	Looks well
		33	8	540	30	78	20	Drainage wound sealed Plasma contains bile
		34	8	540	34	55	21	Plasma contains bile
		35	6	530	36	75	21	Plasma contains bile
		36	6	520	31	65	23	Plasma contains bile
		37	15	500	31	47	25	Plasma contains bile
		38	10	450	32	58	22	Plasma contains bile
		40	7	500	32	62	20	Plasma contains bile
		41	9	540	26	57	23	Plasma contains bile
		42	8	520	26	38	22	Plasma contains bile
		43	6	520	23	55	20	Plasma contains bile
		47	8	530	23	58	18	Plasma contains bile
		48	8	530	28	50	31	Drainage fistula reopened
		49	6	550	24	50	20	Looks well
		50	12	530	29	45	23	Looks well
		51	10	550	32	57	25	Looks well
		52	12	550	26	57	27	Looks well
		53	11	570	30	59	24	Looks well
		54	13	560	30	66	29	Looks well
		55	10	570	30	47	34	Looks well
		56	7	600	30	59	17	Looks well
	9	57	13	580	28	45	30	18 mg. intravenously
		58	10	600	30	50	27	Looks well
		59	18	570	37	65	36	Looks well
		60	14	570	36	62	28	Looks well
		61	17	580	38	62	32	Looks well
		90						Animal killed on this day. No blood changes

interval is greater than 5. Apparently the violence of the preliminary symptoms could not be taken as an index to the prognosis in this group.

There were no changes in the constituents of the blood chemistry investigated whenever the animal was permitted to vomit within five minutes. However, when the emesis interval was greater than 5 the characteristic

changes of mercurial poisoning occurred in the blood chemistry. These changes were sublethal in character whenever the interval was less than 13. (Chart 3.)

The use of milk in the method described above for the animals in Group 2 did not change the length of time required for a lethal dose to be absorbed. All the animals died whenever the interval exceeded 13. It could not be demonstrated that the utilization of milk in any way influenced the symptoms or complications of the toxemia.

In the dogs receiving calcium sulphide as an immediate antidote the same phenomena occurred. Regardless of whether the mercuric chloride was

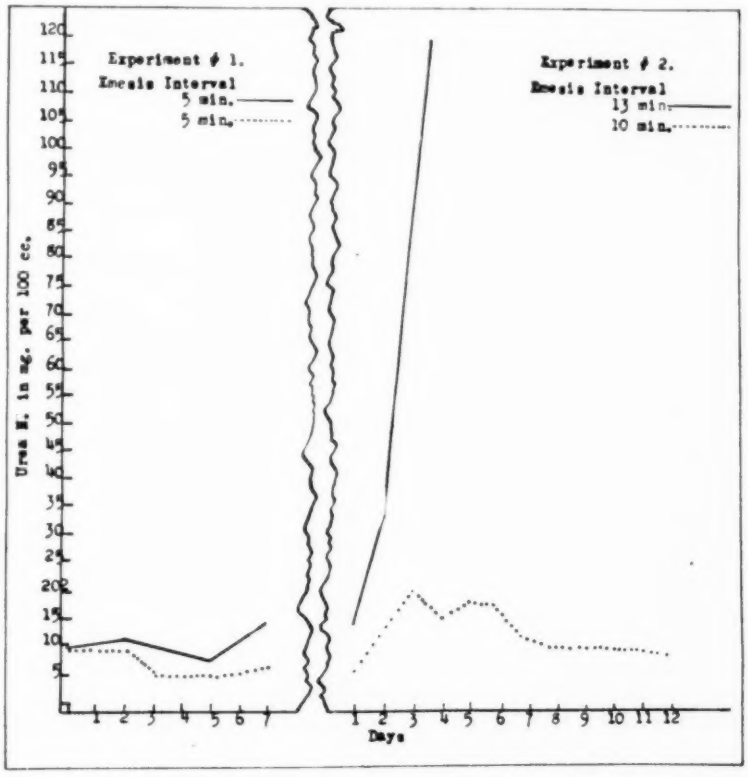

Chart 3.—Blood chemistry of $HgCl_2$ orally; experimental; interval, twenty days.

given in milk or in water solution, the prompt administration of calcium sulphide failed to save any dogs that were not permitted to vomit within the thirteen-minute period.

Of the 10 animals that received gastric lavage with sodium thiosulphate those in which the procedure was delayed for more than thirteen minutes succumbed.

Dye Absorption.—The question of the rapidity of the absorption of the blue dye which is usually used to color the commercial tablet, was investigated. The vomitus was found to be colored blue if emesis occurred within the first ten minutes. Whenever a longer interval elapsed no traces of the

TABLE III

MORTALITY AFTER ORAL ADMINISTRATION OF MERCURIC CHLORIDE. RELATION TO EMESIS INTERVAL

DOG NO.	WT. KG.	DOSE MG.	M.L.D. MG.	EMESIS INTERVAL	REMARKS
1	10	500	262	1 min.	Dog vomited, 7.5 gr. tablet placed in throat dry and washed down with 200 c.c. of water. Vomiting and retching very marked, vomitus contained gross blood. No signs after first day.
2	12	500	300	2 min.	Marked retching and vomiting with severe cramping noted immediately. No signs after first day.
3	12	300	300	5 min.	Animal very sick during the first two days. Marked hematemesis and diarrhea. Blood chemistry showed no significant changes.
4	7	225	175	10 min.	No symptoms and no change in blood chemistry.
5	13	500	325	13 min.	Marked retching and vomiting. Some hematemesis first day. No symptoms thereafter.
6	12	300	300	13 min.	Dog very sick. Died on fifth day.
7	16	500	400	23 min.	No definite signs of intoxication until third day. Death on fourth day.
8	6	225	150	30 min.	Died on the second day, having shown typical findings in the blood chemistry and at necropsy.
9	9	256	225	40 min.	Died on fourth day. Typical changes in blood chemistry.

TABLE IV

CHEMICAL CHANGES IN BLOOD AFTER ORAL ADMINISTRATION OF MERCURIC CHLORIDE RELATION TO EMESIS INTERVAL. AMOUNT OF BLOOD PER 100 C.C.

DOG NO.	WT. KG.	TIME DAYS	DOSE PER KG.	EMESIS INTERVAL MIN.	UREA N. MG.	CHLORIDES Nacl MG.	CO_2 VOL.	REMARKS
1	12	1	24 mg.	5	7	500	42	Looks well
		2			14	490	42	Looks well
		3			21	530	38	Looks well
		4			17	440	36	Looks well
		5			20	500	36	Looks well
		6			18	480	39	Looks well
		7			11	450	42	Looks well
		10			13	470	45	Looks well
		35			14	480	45	Looks well
2	12	1	24 mg.	13	15	580	38	Looks well
		2			33	460	32	Looks sick, refuses food
		3			65	440	22	Sick
		4			116	420	20	Very sick
		5						Died during night
3	9	1	25 mg.	13	17	450	40	Looks well
		2			60	350	25	Not very active
		3			76	300	22	Very sick
		4						Died during night
4	16	1	32 mg.	25	12	450	46	No signs
		3			60	400	30	
		4			84	300	22	
		5						Dog died during night
5	9	1	30 mg.	75	17	400	39	
		2			60	370	28	
		3			87	290	20	
		4						Died during night

dye could be grossly detected. This held true regardless of whether the powder was given dry or in aqueous or in milk solution. The presence therefore of the blue coloration of the vomitus was taken to indicate that emesis had occurred within the first ten minutes after ingestion of the poison, and consequently permitted a very good prognosis with reference to fatality.

Influence of Previous Feeding.—Animals which recovered were subjected to experiments to determine the effect of previous feeding or fasting. It was found that the animal which had previously been fed, vomited spontaneously earlier than the animal which had been fasted for a twenty-four hour period. When the emesis interval was controlled, the presence of food in the stomach did not prevent the absorption of a lethal dose within the thirteen minute period.

Blood Coagulation.—It was incidentally noted during the study of the blood chemistry that blood coagulation occurred much more rapidly during the acute toxemia than before or after it, and that the amount of potassium oxalate necessary to prevent clotting frequently had to be double or triple that which would inhibit it in normal dogs.

Autopsy Findings.—Necropsies were performed upon all animals. The finding of marked emaciation and immediate rigor mortis was characteristic. Hyperthermia was noted in many animals immediately after death. The evidences of salivation and bloody diarrhea were frequently seen.

The brain was yellowish-gray in color, suggesting fatty degeneration. The cortical vessels were dilated and the vessels of the pia-arachnoid were congested. The heart was practically always dilated and usually filled with postmortem clots. The lungs were collapsed, showing marked congestion of the parenchyma and occasionally very marked subpleural hemorrhages. When death occurred immediately following the administration of the drug either by mouth or by vein, intense edema of the lungs was the most characteristic finding. The liver was enlarged, congested, and fatty. Animals which lived for a long time (fourteen days) showed a typical picture of acute yellow atrophy. The spleen was slightly hyperemic but otherwise grossly not affected. The kidneys were large, pale, purple, soft, and congested in these animals. When immediate death took place kidney changes were manifested by large purple swelling. On section the glomeruli were not visible. The medulla was intensely congested and a characteristic pale yellow line ran through the center of the cortex. Examination of the gastrointestinal tract showed the esophagus lined with a brownish mucoid exudate, the stomach contracted with the rugae prominent and presented small petechial hemorrhages. The duodenum was hemorrhagic and ulcerated. The ileum and jejunum were usually clear although the colon was invariably intensely hemorrhagic with marked sloughing of the mucosa.

CONCLUSIONS

1. The blood chemistry of normal dogs was found to be within the limits described by other authors.

2. Blood chemical changes following the intravenous or oral administration of $HgCl_2$ were characteristic.

a. Marked increase in the urea nitrogen.
b. Parallel increase in the nonprotein nitrogen.
c. Decrease in the chloride content.
d. Decrease in the sugar content.
e. Decrease in the alkali reserve.

3. The minimum lethal dose established by Sansum for $HgCl_2$ administered intravenously as well as the anuric dose of this author is confirmed.

4. The mortality following the administration of bichloride orally varied directly as the emesis interval.

5. Blood chemical changes following the oral administration of $HgCl_2$ are proportional to the emesis interval.

6. The mortality following the oral administration of $HgCl_2$ in milk varied directly as the emesis interval.

7. The use of calcium sulphide as an immediate antidote did not influence the rate of absorption of a lethal dose of the poison.

8. The antidotal value of sodium thiosulphate when used as a gastric lavage could not be demonstrated if utilized after a thirteen minute interval.

9. The time required for the absorption of a lethal dose of mercuric chloride dry, in aqueous solution or in milk was approximately thirteen minutes.

10. The time required for the absorption of the blue dye with which the tablets are colored was approximately ten minutes.

11. There was a marked lowering of the coagulation and bleeding time during acute mercurial toxemia.

12. Chemical changes in the blood and the symptoms presented by the animal after the intravenous administration of mercuric chloride were prevented by cholecystostomy.

13. Sudden death following the intravenous or oral administration of large doses of the drug indicated involvement of the nervous system (convulsion), or of the cardiovascular system (shock), or of the pulmonary system (edema of the lungs).

14. The gastro-enteric system, the liver and kidneys were involved almost immediately but death from these causes was usually delayed for several days.

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LABORATORY METHODS

THE USE OF DOGS IN THE ASSAY OF DIGITALIS*

A Comparison With the Results Obtained on Cats

By Charles C. Haskell, J. R. Copenhaver, G. E. Stone, and O. R. Yost, Richmond, Va.

THE important place in therapeutics occupied by digitalis renders it extremely desirable that at least approximate uniformity be secured in the potency of commercial preparations of the drug. The recent report of van Wijngaarden[6] furnished additional evidence as to the unreliability of chemical methods for assay of digitalis and supported the view that attention must still be directed toward physiologic tests in the attempt to standardize the preparations. For some twelve years, the cat method of Hatcher and Brody[3] has been used in this laboratory; an analysis of the results that have been obtained with this method has been published previously.[1, 2] Here, it suffices to state that we are convinced that the method is sufficiently accurate for practical purposes, provided a sufficient number of cats is used in each assay. Unfortunately, it has been our experience that it is difficult to secure cats in large enough numbers at certain times; that our experience is not unique is evident from the statements of other workers. Knaffl-Lenz has recently proposed[4] the substitution of guinea pigs; and, employing a technic similar to that proposed by Hatcher and Brody for cats. Knaffl-Lenz has secured quite satisfactory results by slow intravenous injection of the digitalis solution into the jugular veins of the pigs. Some years ago, Rowe suggested[5] the use of dogs for digitalis assay, but considered that these animals were unsatisfactory, because the toxicity of different samples of digitalis and strophanthus did not show the same ratio on cats and on dogs. With us, for certain reasons, dogs would be preferable to either cats or guinea pigs; dogs can be secured in large numbers; the necessary dissection is simple; and they are cheaper than guinea pigs. In view of the experience as to the large number of cats required to attain approximate accuracy in the assay of digitalis, we suspected that Rowe's conclusions were not justified, because of his failure to use a sufficient number of animals, either cats or dogs, in most of his assays. Having recently tested a sample of tincture of digitalis on a total of 81 cats,[2] it seemed that opportunity was offered of obtaining information of interest by testing the same tincture on a large number of dogs.

In carrying out the assay of digitalis on dogs, the animals were first given 10 mg. of morphine sulphate per kilogram subcutaneously and were then etherized for the operative procedures. Because of ease in handling the

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animals and for the sake of economy in the use of digitalis, small dogs were used in most cases; many of these were full grown; some were quite immature. The tincture (W-26; percolated in 1926), diluted with nine parts of isotonic salt solution, was injected into the femoral vein at the approximate rate of 3 c.c. of the dilution every two and one-half minutes. Artificial respiration was not used, because we feel that this rather lessens the sharpness of the end-point and has no compensating advantage. On the appearance of marked cardiac weakness, the rate of digitalis injection was materially reduced.

A total of 76 dogs received injections of the tincture W-26 in the manner described. It was planned to use groups of six animals each on different days; on two occasions, however, a dog was accidentally killed with ether, consequently, there are eleven groups, each with six, and two groups, each with five individuals. In Table I are given the results of these tests.

The average lethal dose from all dogs in the series was 140.8 mg. per kilogram; the same tincture tested on 81 cats was found to have an average lethal dose of 79.3 mg. per kilogram. The smallest amount of digitalis required to cause the death of a dog was 76.3; the largest, 241 mg. per kilogram; differences greater than these were encountered with cats, where the extremes were 45.3 and 123.2 mg. per kilogram. Of the 76 dogs, the lethal dose for 38 fell below the average and for 38 was above the average.

Considering the averages for the separate groups, the results appear definitely less satisfactory than those obtained on cats: thus, of the 13 dog groups, the averages of only five fell within 10 per cent of the general average; while thirteen of the fourteen cat groups came within this limit. The high group average for dogs, 165.6 mg. per kilogram, was 17.6 per cent above; the low group average for dogs, 121.1 mg. per kilogram, was 14.0 per cent below the general average; as compared with 16.2 per cent above and 9.5 per cent below the general average for corresponding cat groups. In almost every respect, it seems that dogs are less satisfactory than cats for the assay of digitalis.

Several factors may have been responsible for the comparatively poor showing secured by the use of dogs in testing this sample of digitalis. As already mentioned, the size of the animals used was not uniform. The average weight of the dogs was 5.85 kilograms; the extremes were 2.2 and 16.0 kilograms. The average lethal dose for 42 dogs weighing less than 5.85 kilograms was 142.8 mg. per kilogram; that for 34 dogs weighing more than 5.85 kilograms was 135.6 mg. per kilogram. This suggests the possibility that the weight of dogs may have an influence in causing variations in susceptibility to digitalis greater than was found to be the case with cats.[2]

In 11 instances, note was made that the animal used was a puppy; the average lethal dose for these 11 puppies was 148.0 mg. per kilogram. It might be inferred from this that young animals have a higher resistance to digitalis than older dogs possess; however, the limited number of observations scarcely justifies this.

Although our experience indicates that dogs are distinctly less satisfactory than cats for the assay of digitalis, it is obvious that the employment of a sufficiently large number of dogs for each assay will afford results not far

TABLE I

ASSAY OF TR. W-26 ON DOGS

DATE	DOSE OF DIGITALIS MG. X KG.	AVERAGE
Sept. 29	133.2	
	142.0	
	141.9	133.3
	122.8	
	133.3	
	114.4	
Oct. 4	150.2	
	97.3	
	122.7	146.2
	170.0	
	241.0	
Oct. 5	144.4	
	150.0	
	157.9	136.4
	119.6	
	100.0	
	146.4	
Oct. 10	146.2	
	122.2	
	183.1	137.9
	133.3	
	124.4	
	118.5	
Oct. 12	126.0	
	133.0	
	125.8	126.4
	160.0	
	101.5	
	112.4	
Oct. 25	141.5	
	156.9	
	146.1	142.7
	150.8	
	140.5	
	120.6	
Oct. 26	166.6	
	115.4	122.9
	76.3	
	128.8	
	127.4	
Oct. 31	194.1	
	133.3	
	124.0	158.2
	162.6	
	142.4	
	192.8	
Nov. 1	121.5	
	142.7	
	118.4	121.1
	105.8	
	113.4	
	125.1	
Nov. 8	113.3	
	160.0	
	162.0	143.6
	143.0	
	150.0	
	133.3	
Nov. 22	142.2	
	194.7	
	145.0	165.6
	149.3	

TABLE I—CONT'D

DATE	DOSE OF DIGITALIS MG. X KG.	AVERAGE
	151.3	
	211.2	
Dec. 6	142.6	
	150.0	
	113.1	135.9
	168.2	
	132.4	
	109.3	
Dec. 14	163.1	
	135.1	
	151.6	149.6
	137.2	
	140.6	
	169.8	
Average of total	140.8	

from the probable potency of the preparation tested. If, however, it is true that the relative toxicity of different samples of digitalis is not the same on cats and on dogs, doubt would be cast on both methods of testing digitalis. The lethal dose of Tr. W-26 for dogs is 1.77 greater than that for cats; by reference to figures obtained from previous tests, it has been possible to estimate the relative toxicity of other digitalis preparations on dogs and on cats; the results of such estimation are given in Table II.

TABLE II

RATIO OF TOXICITY OF QUABAIN AND OF TINCTURES OF DIGITALIS AS DETERMINED ON CATS AND ON DOGS

PREPARATION	LETHAL DOSE IN MG. X KG.		RATIO
	DOGS	CATS	
Ouabain	0.178	0.0991	1.80
	(8)*	(27)*	
Tr. W-26	140.8	79.3	1.78
	(76)*	(81)*	
Tr. 33	126.8	76.5	1.66
	(6)*	(12)*	
Tr. 36	74.3	41.3	1.80
	(5)*	(12)*	
Tr. 51	145.3	83.1	1.75
	(7)*	(14)*	

*Number of animals used.

It is obvious from the figures in Table II that the ratio of toxicity for the different samples of digitalis bodies is so near the same, although a comparatively smaller number of animals was used in some cases. It would appear that the lethal dose of a digitalis body is consistently higher for dogs; and, so far as reliance may be placed on this limited number of observations, it is possible to translate the results of an assay of digitalis on dogs to the more familiar "cat units" by dividing the lethal dose for the dogs by a figure around 1.75.

It may be concluded that, so far as accuracy is concerned, cats are superior to dogs for the assay of digitalis. Nevertheless, a fairly satisfactory assay of digitalis may be carried out by the utilization of from six to ten dogs in each test. So far as the limited number of samples compared justifies infer-

ences, it may be assumed that the relative toxicity of different digitalis preparations is the same on dogs and on cats.*

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MEDICAL COLLEGE OF VIRGINIA.

THE DETERMINATION OF AMMONIA BY AERATION†

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URBANA, ILLINOIS

IN THE determination of ammonia by the aeration method of Van Slyke and Cullen (1916) one meets with the inherent difficulties of the use of very dilute acids and alkalies; namely, the satisfactory standardization of these reagents, their keeping qualities, and the judgment of the end-point of the titration. In the course of some investigations requiring the aeration method for the determination of ammonia we have introduced two procedures, one of which eliminates the use of the standard alkali and the other increases the accuracy of the titration. Our method is as follows:

The apparatus of Van Slyke and Cullen is set up exactly as described by them, excepting that in the receiving tube 40 c.c. of a 4.5 per cent boric acid solution, measured approximately, is used instead of 25 c.c. of N/50 hydrochloric or sulphuric acid. The aeration is then carried out at an initial rate of between three and four liters of air per minute for five minutes and then at a rate of twelve liters for twenty-five minutes. The ammonia absorbed by the boric acid is titrated with N/56 hydrochloric acid using methyl orange or bromophenol blue as the indicator, the end-point being accurately determined by comparison with the following arrangement of tubes providing a standard which is readily reproducible in shade and intensity of color. The titration is conducted in a four-holed comparator block. One side of the block has two Van Slyke and Cullen aeration tubes containing 80 c.c. of water. With methyl orange as an indicator one of these tubes contains 0.5 c.c. of 0.04 per cent methyl orange and a drop of concentrated hydrochloric acid; the other tube contains 1.5 c.c. of the methyl orange solution and one drop of 10 per cent sodium hydroxide. If bromophenol blue is used each tube contains 80 c.c. of water, 1 c.c. of 0.04 per cent bromophenol blue, and is respectively acid and alkaline as above. The other side of the block holds an aeration tube of distilled water and the experimental tube with 2 c.c. of the indicator solution. As the end of the titration is approached the experimental

*Tests carried out since the completion of the experiments described above suggest that there is apparently a seasonal variation in the resistance of dogs toward ouabain. Certainly, our statement that the fatal dose of this substance for dogs is 0.178 mg. per kilogram requires modification; during the past summer it has been found to be 0.139 mg. per kilogram. This would give an entirely different ratio for ouabain and for digitalis on cats and on dogs.

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tube is diluted to approximately 80 c.c. with water neutral to, but not containing, the indicator in use. The tubes in the comparator block are illuminated by the light from an ordinary 100 watt lamp reflected from a sheet of white paper.

DISCUSSION

Winkler (1913) proposed the use of boric acid to receive the ammonia in the determination of nitrogen by the Kjeldahl method and the direct titration of the ammonia. Adler (1916) and Scales and Harrison (1919) found the method entirely satisfactory. The procedure effects a saving in the time of measuring the solution for the ammonia absorption, reduces the number of readings of burettes from two to one, eliminates the use of the comparatively unstable standard alkali solution, provides a large capacity for ammonia absorption, and conserves the standard acid solution employed, as the amount used is equivalent to the nitrogen involved. Kober and Graves (1913) attempted to apply boric acid to the fixation of ammonia in aeration. They reported that ammonia escaped from boric acid and could be detected in a second absorption tube by nesslerization. We have conducted a similar experiment and compared the ammonia holding efficiency of 40 c.c. of 4.5 per cent boric acid with that of 25 c.c. of N/14 hydrochloric acid. The aeration rates as given above were employed. Twenty-five c.c. of N/56 hydrochloric acid were placed in a second absorption tube. At the close of the aeration the boric acid tubes were titrated with N/14 hydrochloric acid and the second absorption tubes of each series were transferred to 100 c.c. flasks, treated with 10 c.c. of Nessler's solution, diluted to the mark, and compared with standards. The results of a typical experiment as recorded in Tables I and II show that *in aeration a 4.5 per cent solution of boric acid is apparently just as effective in absorbing and retaining ammonia as N/14 hydrochloric acid.* The experiment also shows the capacity of 40 c.c. of boric acid to be far in excess of the amounts of ammonia commonly determined by aeration.

TABLE I

AERATION INTO 40 C.C. OF 4.5 PER CENT BORIC ACID

NUMBER OF SAMPLE	NH_3–N PRESENT MG.	TITRATION NH_3–N MG.	NESSLERIZATION NH_3–N MG.
1 A.	5.00	5.00	less than 0.010
2 A.	10.00	10.00	less than 0.025
3 A.	15.00	15.00	less than 0.025
4 A.	20.00	20.00	0.030
5 A.	25.00	25.00	0.050

TABLE II

AERATION INTO 25 C.C. N/14 HYDROCHLORIC ACID

NUMBER OF SAMPLE	NH_3–N PRESENT MG.	NESSLERIZATION NH_3–N MG.
1 B.	5.00	less than 0.010
2 B.	10.00	less than 0.025, and less than 2 A.
3 B.	15.00	less than 0.025, equivalent to 2 B.
4 B.	20.00	less than 0.050, but greater than 4 A.
5 B.	25.00	0.050

The precision of the method is revealed by the following experiment. Various dilutions of pure ammonium sulphate were made by one of us and the series of "unknowns" analyzed by the other. The results appear in Table III.

TABLE III

NUMBER OF SAMPLE	NH_3–N PRESENT MG.	BROMPHENOL BLUE NH_3–N FOUND MG.	METHYL ORANGE NH_3–N FOUND MG.
1	3.24	3.16	3.18
2	5.32	5.21	5.18
3	0.90	0.87	0.90
4	4.98	4.94	4.95
5	6.31	6.30	6.30
6	1.49	1.49	1.51

The use of bicolor standards in a comparator block for the determination of titration curves was suggested by Gillespie (1920). His method, however, differs from ours in that he uses only a part of his solution for comparison and that he was interested in the actual P_H of his solutions. We have the entire solution in the comparator block and make use of the bicolor system only to fix an arbitrary, readily reproducible end-point, not necessarily of known P_H.

We have found that caprylic alcohol interferes with nesslerization for the determination of the traces of ammonia carried through boric or hydrochloric acid. By substituting a drop of phenyl ether (Lee, 1917) in each tube we have entirely eliminated the interference and have found it to be just as effective an antifoamer as caprylic alcohol. Phenyl ether is not dispersed by aeration or stirring as readily as caprylic alcohol and so does not interfere with observing the true color of the indicator due to any cloudiness of the solution. It can be obtained for one-tenth the cost of the alcohol.

For convenience the entire apparatus with the exception of the light source is arranged on a single iron stand with the comparator block raised to eye level. Stirring is effected by means of a footed glass rod operated by a string pulled through a short, inverted U-tube supported above the comparator block.

It is necessary in our procedure to control the concentration of the indicator. An alternative to the method of dilution as given above is to titrate with N/56 hydrochloric acid containing 10 mg. of methyl orange or bromophenol blue per liter. Using this method we use the bicolor standards as given above but only 1 c.c. of the indicator solutions in the experimental tube. Any wash water added should be neutral to the indicator in use and contain 10 mg. of methyl orange or bromophenol blue per liter.

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THE CLINICAL VALUE OF THE SALIVARY UREA INDEX*

By Clair L. Stealy, M.D., San Diego, Calif.

SINCE determinations of blood urea nitrogen and creatinine are very important factors in the estimation of renal impairment, an easily applied and accurate method of estimation which could be used routinely or as a simple indicator to sift out cases of renal impairment with retention for more careful study would be of great value in the everyday practice of medicine. Our present methods are invaluable but the training and equipment necessary prohibit their use by all except the larger institutions and laboratories. Also, while urinary findings are simple indicators of kidney impairment, we do not like to subject every patient whose urine contains albumin or casts, or shows a fixation of specific gravity, to these more complicated tests. Therefore, when Hench and Aldrich[1] published their findings of a simple method for using the salivary urea nitrogen determination as an index to the blood urea nitrogen, it was seized upon as at least an indicator which would sift out cases of renal impairment with retention for more detailed study.

Marshall and Davis[2] and Myers and Fine[3] have demonstrated that urea nitrogen is diffusible in all body secretions and that its concentration in these secretions checks within normal limits with that of the blood urea nitrogen, both in normal individuals and in those with renal impairment. Little is said, however, as to the time element and pathologic conditions which may influence this diffusibility of urea nitrogen into body tissue.

Upon these facts as established by Marshall and Davis, and Myers and Fine, and other workers, Hench and Aldrich[1] started experiments on a secretion which was easily obtainable, namely, saliva, to determine whether or not its urea nitrogen content could be used as an index to the blood urea nitrogen, and therefore to renal impairment. Using the urease method they concluded that the average urea nitrogen content of the saliva is approximately 80 per cent that of the blood. Then, following the work of Friedlander, based on the mercury-combining power of saliva they developed a very time-saving and inexpensive technic for the determination of the salivary urea nitrogen which they showed could be used as an index to the blood urea nitrogen.

For our own satisfaction, we have checked this method in our laboratory, taking the cases as they presented themselves, regardless of diagnosis, to determine whether or not the blood urea nitrogen as determined by the salivary urea index and the actual blood urea nitrogen checked within sufficiently close limits to be used clinically, and whether or not the limits given by Hench and Aldrich as indicative of retention held for both normal and pathologic cases, for it has seemed to us that any condition which would disturb the functions of the rest of the body might also disturb the functions of

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the salivary gland or the diffusibility of urea nitrogen through this gland, and not necessarily in the same ratio.

Hench and Aldrich say that "when a salivary urea index below 50 is obtained, there is, in nearly 90 per cent of the cases, no urea retention in the body. There is a 10 per cent possibility that mild retention exists, but not in any case does it exceed the blood urea value of 60 mg.," and that "when a salivary urea index above 50 is obtained, there is in 91 per cent of the cases definite urea retention, and only a 9 per cent possibility that there is no retention."

In our series, 75 estimations of normal and pathologic cases were made. The probable blood urea nitrogen as estimated from the salivary urea index and the actual blood urea nitrogen checked closely in the normal cases, as shown in Table I. The pathologic cases showed large and varied discrepancies between the probable blood urea nitrogen and the actual blood urea nitrogen. For convenience these cases have been divided into groups according to diagnosis and the findings tabulated in Table II which shows the number checking within normal limits, the number in which the actual blood urea

TABLE I

PROBABLE AND ACTUAL BLOOD UREA NITROGEN DETERMINATIONS IN NORMAL CASES

SALIVARY UREA INDEX	PROBABLE BLOOD UREA N. MG. PER 100 C.C.	ACTUAL BLOOD UREA N. MG. PER 100 C.C.
31	10.33	11.8
32	11.76	11.8
30	8.9	9.8
33	14.0	15.0
34	14.62	15.0
34	14.62	15.0
34	14.62	15.0
32	11.76	12.4
33	13.19	12.0

TABLE II

PERCENTAGE ERROR BETWEEN PROBABLE AND ACTUAL BLOOD UREA NITROGEN DETERMINATIONS IN NORMAL AND PATHOLOGIC CASES

DIAGNOSIS	NORMAL	DIABETES	HYPERTROPHY OF PROSTATE	NEPHRITIS	MYOCARDITIS	MISCELLANEOUS
Number of determinations	9	17	16	10	14	10
No. in which probable checked with actual blood urea N.	9 (100%)	1 (10%)	--	--	3 (21%)	5 (50%)
No. in which probable was above actual blood urea N.	1 (11%)	2 (20%)	4 (25%)	7 (70%)	6 (43%)	--
Per cent error	0 to 0.9%	29 to 183%	10 to 71%	23 to 180%	54 to 189%	--
No. in which probable was below actual blood urea N.	8 (89%)	14 (70%)	12 (75%)	3 (30%)	4 (36%)	5 (50%)
Per cent error	0 to 1.4%	22 to 72%	10 to 200%	31 to 50%	14 to 48%	18 to 50%

nitrogen was below that estimated from the salivary urea index, the number in which the actual blood urea nitrogen was higher, and the percentage error in each group. In two of these cases the salivary urea was so high that it ran above the calibration of the flask so that the estimated blood urea nitrogen was 140 and 120 mg.; the actual blood urea nitrogen in these cases was 75 and 58 mg.

TABLE III

PROBABLE AND ACTUAL BLOOD UREA NITROGEN DETERMINATIONS IN CASES OF DEFINITE RETENTION

SALIVARY UREA INDEX	PROBABLE BLOOD UREA N. MG. PER 100 C.C.	ACTUAL BLOOD UREA N. MG. PER 100 C.C.	DIAGNOSIS	REMARKS
45	30.35	27.0	Prostatitis	
45	30.35	27.8	"	P. S. P. 9½% in 2 hr.
34	14.62	20.7	"	
38	24.34	36.0	"	
31	10.33	15.0	Pyonephrosis	P. S. P. 33% in 1 hr.
46	31.78	40.88	Diabetes	P. S. P. 9½% in 2 hr.

Table III shows the determinations made in the six cases of renal impairment with definite urea nitrogen retention. The percentage error between the probable and actual blood urea nitrogen determinations falls within allowed limits but in all of these six cases the salivary urea index was less than 50 which according to Hench and Aldrich would have indicated no urea nitrogen retention, allowing for a 10 per cent error. These six cases constitute 10 per cent of the 54 having a salivary urea index of less than 50, but we cannot agree that a method which would allow an error in six out of 54 cases is sufficiently accurate for clinical use.

CONCLUSIONS

1. In the normal individual, as shown by Hench and Aldrich and by ourselves, the probable blood urea nitrogen as estimated from the salivary urea index, and the actual blood urea nitrogen are in very close relationship.

2. In pathologic cases there is a wide variation between the probable blood urea nitrogen as indicated by the salivary urea index and the actual blood urea nitrogen. Apparently this variation follows no definite rule or pathologic condition, and this indicates, we believe, that with the pathology, other factors enter in such as the rate of diffusibility of the urea or a change in the tissue structure of the salivary gland. In some cases the saliva showed the presence of an albuminous substance which did not precipitate on boiling and apparently was at least partly responsible for the wide variations in the probable and actual blood urea nitrogen determinations since it was present in all but two cases showing wide variation.

3. The figures given by Hench and Aldrich as indicating the limit of normal urea nitrogen content of the blood are too high and may include many cases of actual urea nitrogen retention. The limits of tolerance are too great for accurate clinical work.

4. Finally, we conclude that the use of the salivary urea nitrogen determination as an index to the blood urea nitrogen content, as worked out by Hench and Aldrich can neither be used as a substitute for blood chemistry nor as an accurate indicator or selector of cases to be subjected to more complete tests.

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GINGIVITIS, III*

Laboratory Methods for Study

By Robert A. Keilty, M.D., Washington, D. C.

THE purpose of this paper is to report a method for the laboratory study of the gingivae and their sulci. It is one of a number of papers giving the details of different phases of this subject. The method, as outlined, is one used in the study of gingivitis, previously reported and by practical application may be adapted to the needs of the modern dentist as an assistance in diagnosis and treatment. The laboratory worker can be of tremendous help to the dentist by determining the character and amount of infection and infestation of the gingivae in a given case.

In the past this very important bacterial culture field, the gingival sulcus, has been neglected by the laboratory, except by those men who have been especially interested in either obtaining material for a special study or in attempts to determine the etiology of "pyorrhea" (a term discarded for the more inclusive one of gingivitis). It will be necessary for clinical bacteriologists to familiarize themselves with this nest of infection and infestation and then to cooperate and study the problem with the assistance of a good modern dentist. It will be necesary to hold an open mind until the last of the problem has been conquered and nothing will be gained by fixed and finished opinions either as to what has been discarded in the past, what is now controversial, or what may be problematic for the future. I have said finis to the subject several times during the past eight years but now, more than ever, regard it of prime importance. It is one that bids fair, the sooner it is recognized in its early stage, to counteract much future trouble for a large group of the people. It is necessary then for the laboratory worker first to become thoroughly familiar with the field, to educate some good dentist or group with whom he may work and then both cooperate for the benefit of the individual patient as

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well as for the solution of the problem as a whole. This group study appeals to the internist, and with his complete cooperation the results obtained in the selected group of gingival focal infections are often striking.

In the study of infection and infestation of gingival exudates, I have made use of fresh and stained smears, various types of media, and pathologic sections where possible. When the patient is referred for the first examination, a complete report, made by the dentist, of the condition of the teeth and a complete dental x-ray is of great assistance. The report should call attention to mechanical dental conditions responsible for traumatic areas, the general dental survey and any teeth which are so-called dead or devitalized. It is important to know just what, when, and how long treatment has been directed at the gingivae. The dental x-ray will immediately call attention to the degree of deep changes about the roots of teeth. While the history is of great aid, the character of the bacteriologic examination is entirely in the hands of the laboratory man, and he should make his own survey as he sees the field. He should not by any means merely carry out a routine order, examine for Vincent's spirillum or examine tooth No. 5 for the presence of streptococcus.

In the examination make a complete survey of the mouth noting the conditions briefly and describing in some detail the pathologic picture of the gingivae. This should include a brief description of the mouth as a whole, dentures, bridges, crowns, fillings, and dental hygiene. *It should not be and is not the field or duty of the clinical pathologist to note the mechanical features of the mouth, only in so far as they are related to the problem of infection and in so far as their correction is a means to an end in treatment.* Bridges, crowns, and dentures are secondary etiologic factors or negative phases in gingivitis and must be corrected before the infectious factors can be conquered. This is the problem of the surgical dentist but here cooperation with the laboratory is not only valuable but absolutely necessary. It is pretty hard on a patient who has recently had from eight hundred to fifteen hundred dollars worth of dental mechanical work, to be told that this must be sacrificed before a complete infectious problem can be controlled, but it must be done. This is an economic problem. I have recently seen two such cases, one of iritis and one of polyarticular arthritis where the correction of infectious factors compelled an entirely new dental mechanism. This is an educational problem on the side of the dentist and a big responsibility on the side of the pathologist. The responsibility for the latter is, first, the accurate recognition of existing conditions, and second, judgment in making recommendations.

It is a responsibility which should be divided between the dentist and the laboratory, since it is intimately concerned with dental mechanics and infectious factors. The sooner the laboratory takes an active interest in the dentist's problems the sooner the unnecessary sacrifice of teeth and life will be stopped, and the sooner the unnecessary trouble and expense of several dentures will be controlled by one proper outline from the beginning. Speaking of loss of life, hardly a month goes by that we do not hear of a case of cellu-

litis, septicemia, and death following a simple dental extraction. This sacrifice of life can be greatly reduced if not absolutely controlled when the infectious factors in the given case are studied and corrected beforehand. This is a statement based upon much experience with infection not corrected following dental operations, and without infection when adequately corrected following extensive dental surgery. The recognition of and preoperative treatment for gingival infections, reduces the postoperative reactions to a minimum and allows extensive surgery which would otherwise be impossible in the presence of infectious factors.

Following the survey of the mouth as a whole, the physical condition of the gums should be noted and if one follows the pathologic outline as described,[1] it is surprising how much can be seen. Especial attention is directed at the sulcal exudate with its character as a whole and with especial note about teeth under special consideration. This is an examination first of the field as a whole and second of individual teeth and their infectious factors. The x-ray picture of a complete mouth before one at this examination is most valuable in directing attention to definite areas.

The bacteriologic character of gingival sulcal infection may be obtained by examination of smears, cultures, and tissue. The smears will give a great deal of information, and fresh smears will give more than stained smears. The dark-field will permit character studies of individual organisms, but I do not believe it is necessary for routine use. The method of obtaining material and the examination of smears has been previously outlined.[2] Study the general character of the infection present by a smear of a number of sulci and all sulci in the mild cases and follow this by examination of individual areas for special study. Three or four preparations may be necessary, the important point is not to miss anything and to make notation of all types of infection recognizable. The fresh smear is made in warm physiologic salt solution and the material is collected by a small loop 22 gauge chrome nickel wire in a holder. We have tried instruments, swabs, tooth picks, and capillary tubes but nothing is more convenient than the chrome nickel wire. Examine fresh smears immediately while they are warm (a warm stage is not necessary) under the high dry power, 4 mm. objective. Make a notation of all types of bacteria and protozoa seen and indicate their prevalence by degrees of 1, 2, 3, 4. This is a very convenient record especially for purposes of treatment checkup. Fresh smears are made on flat glass slides with a coverslip. Concave slides are not necessary but may be used. I have recently been making concave slide cultures cemented with vaseline and paraffin and have found them convenient for incubator purposes.

Stained smears have not been found as instructive as the fresh smears, but are valuable in individual and special studies or for the predominance of bacterial types. The fresh smear may be used for the stained preparation by removing the cover glass, drying and fixing and staining by any method desired; Gram is one of preference. In the study of protozoa I make use of Schaudinn fixative and hematoxylin staining but this is not necessary for routine examination. All in all, the fresh smear may be relied upon routinely

to give all the information necessary from the smear standpoint. It is rapid and there is no staining time delay. Where one sees a number of cases routinely, the factor of time is important. On the other hand when one begins the study of sulcal exudate, every method of examination will be found instructive.

In my paper last year[2] I said that the routine use of cultures was valuable but not necessary owing to the work entailed in examining a large number of cases. I would like to reverse this statement and now include the use of at least a blood agar plate and an anaerobic tube for the routine culture examination. This will entail a considerable burden where the daily examinations amount to any number of cases, but I believe the information obtained is worth the effort. Up until last year cultures were made in individual cases and in group studies but during the year I have routinely cultured every case on at least one blood agar plate and an anaerobic oxygen tension tube modified after Rosenow. This will be necessary until the bacteriologic flora of gingival sulci and its relationship to different types of gingivitis has been definitely established. That there is a relationship would seem to be borne out. There is no question of the advantage of cultural studies for preoperative cleanups. Animal or human, blood agar plates will grow all the coccal and bacillary flora and the same plate may be used for inoculation of several areas. We are still struggling with an adequate method for the cultivation of spirilliform organisms and have nothing to offer more than is in the literature at this time.

The study of gingival tissue cannot be carried on routinely owing to the obvious difficulty of obtaining material. It can be studied in all operative cases, and frozen sections with bacterial stains are very instructive. If the suggestion of Neuman, which I think is entirely too radical, that is, the stripping down of the gingivae of the entire mouth and allowing it to heal by granulation, was ever carried out, tissue would be available but I do not believe this will ever be the case. At this stage of our knowledge it is not necessary to study tissue except for special diagnoses such as granuloma, tumor, or tuberculosis.

Another appeal is made for the careful laboratory study on a large scale of gingival infections and their early recognition with the possible prevention of a trail of manifestations undoubtedly having their origin in the focal infection or portal of entry of a gingivitis. The magnificent work of Rosenow and many others has taken the subject of focal infection entirely out of the realm of faddism and possibility, and it remains for the future to detach it from the uncertainties now existing and firmly attach it to the certainties with which it is definitely and undoubtedly connected. The literature is filled with volumes and unfortunately many of the authors seem to have little or no first-hand information upon their subject. For this reason it is hoped to add in this series of papers some foundation to the clinical experience of the internist and modern dentist by a laboratory correlation.

CONCLUSIONS

This paper deals with a routine method for the examination of gingival exudates with the idea of determining their bacteriologic characteristics.

An appeal is made for a more widespread cooperation between the laboratory examination and the modern surgical dentist.

The field belongs to both the clinical laboratory and the dentist, and there is the nicest chance for a cooperation which is worthy of any effort, since the results accruable to the welfare of the patient are much worth while.

The examination should take into consideration the general character of the mouth, the teeth, the pathologic pictures of the gums, the gingival sulci, and the x-ray picture with special reference to the periodontal areas.

The gingival exudate is best examined by fresh smears and cultures. Stained smears and dark-field may be used for special studies.

The gingival exudates should be examined by culture and the use of a blood agar plate and an anaerobic oxygen tension tube will determine the coccal and bacillary forms.

The spirilliform organisms and protozoa are best determined by smear examination, and a satisfactory culture medium for these forms cannot be offered at this time.

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APPARATUS FOR SHAKING BLOOD COUNTING PIPETTES*

BY ROY F. FEEMSTER, M.D., D.P.H., NEW ORLEANS

IN OUR experimental work it has been necessary to make numerous blood counts on animals and consequently the shaking of the pipettes became quite burdensome. Because of this fact we were led to devise a simple apparatus to do this shaking, and it has proved very useful. Moreover our counts have become more accurate with the use of it. We obtain much better distribution of cells, for we tended to reduce the time of shaking when we had to undergo the discomfort of having the points of the pipettes digging into our fingers a number of times in succession.

The apparatus consists essentially of a light thin piece of poplar board to which the pipettes are securely fastened, and which is rapidly shaken by a small electric motor in such a way as to imitate the usual movements of the hand. Fig. 1 will convey a sufficiently clear idea of the essential features that very little description is perhaps necessary.

Fig. 1-*A* shows the method by which the pipettes are secured to the apparatus. A small wooden strip, pierced by a number of holes just large enough

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to admit the shaft of the pipette without allowing the bulb to pass through, is nailed to one side of the moving board. A row of metal clips, illustrated by Fig. 1-*B*, hold the pipettes at the other side of the board. A rubber band, held at one end by a nail driven in the board, securely presses against the tip of the pipette and keeps the fluid from running out of the bulb during the shaking.

The board carrying the pipettes should be made as light as possible, as the lighter the board the less the vibration. The board is shaken by a bend in a shaft made of heavy wire to which a pulley is fixed. This pulley is

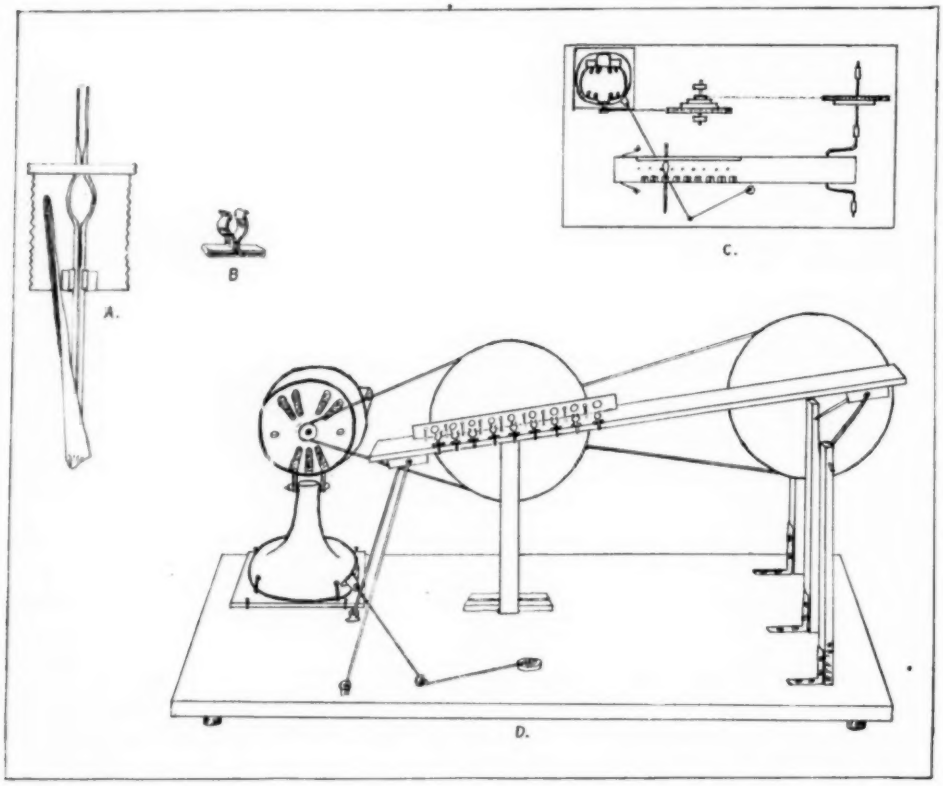

Fig. 1.

turned by a belt coming from a system of pulleys turned by another belt from the electric motor, the whole system being so adjusted that the moving board makes about 300 complete excursions per minute.

We made the pulleys we needed from white pine a little over a quarter of an inch in thickness. Circles of the desired size were cut out with a coping saw and a hole just the size to fit snugly on the shaft of the electric motor was drilled in the exact center of each. They were then mounted on the motor and allowed to turn rapidly, the groove in the circumference then being cut with a sharp instrument, just as on a turning lathe. However, metal pulleys can often be found about a laboratory on an old piece of apparatus.

Figs. 1-*C* and *D* give two views of the arrangement of the parts of the

apparatus. The motor should be fixed to a thin rectangular board which is kept from turning by two nails in front and two behind. The board can still be moved toward and away from the pulleys, which allows the tension of the belt to be adjusted. The weight of the motor usually keeps the board from slipping, but if the belt persists in becoming too loose a spring may be necessary to pull the motor back and keep the proper tension.

A great deal of the vibration can be eliminated by mounting the board, to which all of this apparatus is fixed, upon four rubber legs made by driving nails through rubber stoppers, sinking the heads of the nails into the rubber a fraction of an inch.

A TENDON CLAMP FOR MEASUREMENTS OF MUSCULAR TENSION*

By Enoch Karrer, Ph.D., and H. C. Stevens, Ph.D., M.D., Cleveland

IN MEASUREMENTS of muscular tension, the muscle is attached to the dynamometer or weight by means of its tendon. The connection is usually made by inserting a hook through the tendon. This method is objectionable for the reason that a hook tends to slip, unless special precautions are taken, and, as tensions become great, the hook cuts into the tissues. In certain prob-

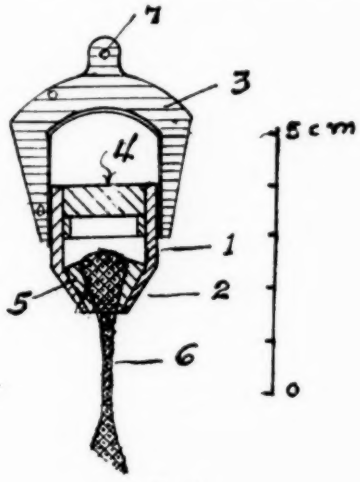

Fig. 1.

lems in which exact measurements of muscular tension are required, such as the problem of the pluri-segmental innervation of muscles, the maximal force of the muscle is exerted with a very slight shortening of the muscle. One-tenth of a millimeter may represent many grams. It is important, therefore, to be able to connect the tendon to the dynamometer in such a manner that there will be no yielding due to slipping or cutting when the muscles exert

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their maximal force. To meet the need for a mechanical mode of attachment which will neither slip nor cut the tissue, a tendon clamp has been devised.

This consists of a small brass cylinder, 1, which is straight for a portion of its length and conical on one end, 2. It has attached to it a yoke, 3, built of thin sheet metal in the fashion of a structural T-beam to give strength with lightness. If thought desirable small holes may be drilled in the yoke for suspending the tendon holder in the horizontal position in case the passive extension of the muscle is not sufficient to so maintain it. This yoke has a hole, 7, for attaching to the dynamometer or counter weight system, by clevis or other means. In the straight end of the small brass cylinder just mentioned, is a nut, 4, which may be unscrewed and removed entirely while the tendon is being inserted. There are two conical aluminum jaws, 5, the two halves of a cone, which fit into the conical end of the brass cylinder, 2. The tendon, 6, which is desired to be clamped is inserted through the hole in the conical end of the brass tube and the aluminum jaws, 5, are inserted from the rear which is open after the removal of nut, 4. This latter nut is then inserted and screwed down tightly against the distal surface of the bone on which the tendon terminates. We have found that rather than screwing nut, 4, onto the tendon, the insertion of a stiff rubber block which bears against the tendon and against the brass nut, 4, is more satisfactory in that it cannot mutilate the attachment of the tendon. It is obvious now that the tendon is securely clamped over a considerable portion of its surface between the aluminum jaws which have a tendency to tighten the greater the force which acts upon the tendon.

A METHOD FOR STAINING CONNECTIVE TISSUE MAST CELLS*

By Michael Levine, Ph.D., New York City

IN STAINING paraffin sections of the parathyroid gland of human subjects and various domestic animals, I have used a variety of combinations of acid, neutral, and basic dyes to bring about a differentiation of the various cellular structures in this gland. A large number of stains, consisting of single dyes, and combinations of dyes were used. Among the latter was found a combination of thionin and orange G which when applied to sections of the parathyroid gland produced a marked contrast between the connective tissue and mast cells, so as to set off the latter with great clarity. The readiness with which the staining process is accomplished and the definiteness of identification of the mast cells are such that a brief description of the technic employed and the results obtained may be of interest to those engaged in investigations connected with mast cells.

There are at present a number of well established methods of staining used in the study of connective tissue mast cells. With Ehrlich's discovery of mast cells in 1877 came the use of dahlia or its synonyms as a suitable

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stain for the study of these cells. Bergonzini (1891) studied the connective tissue of the mouse, rabbit, guinea pig, and frog, after staining in a combination of dyes consisting of a basic dye, methyl green and two acid dyes, namely, fuchsin and orange G. Pappenheim's (1899) methyl green pyronin stain is well known as a very satisfactory preparation for staining mast cells. Schridde (1905) stained with anilin water-acid fuchsin and counterstained with an alcoholic solution of picric acid. This method gives very clear figures when the combination of stains is properly used. His figures are clear and show not only the granules of the cells sharply contrasted against the color of the cytoplasm but also the nuclear content is clearly differentiated. Schaffer (1907) used three stains, toluidine blue, methylene blue, and thionin which are recognized as good basic stains for mast cells. Perhaps one of the most popular stains among laboratory workers is the polychrome methylene blue of Unna (1910). Krause (1927) gives a very comprehensive review of staining methods used in the study of all types of plasma cells.

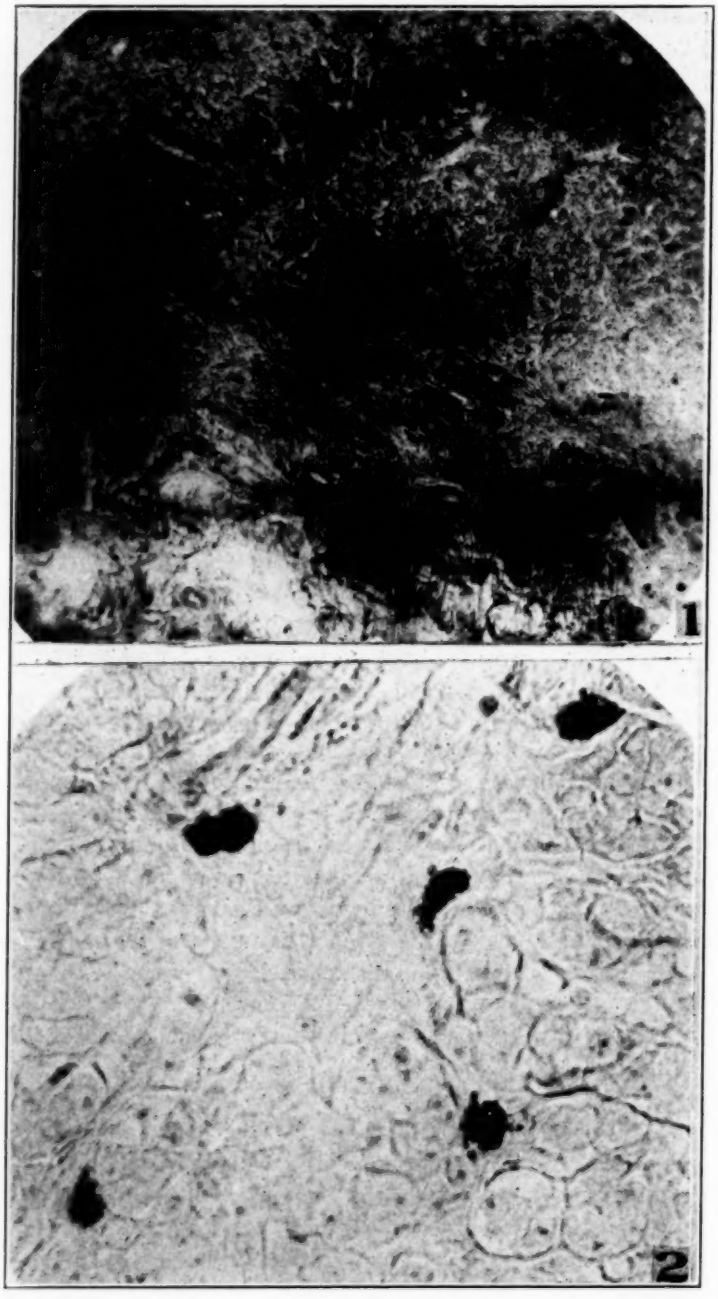
As mentioned above, I tried to stain paraffin sections of the parathyroid gland of various animals including man and I found that while the old stains are useful, they frequently fall short of their purpose. Differentiation of the various cellular elements is frequently lacking. Tissue elements other than the mast cells take up the dyes in the same degree and with the same rapidity so that recognition of the mast cells is difficult. Mast cells with few granules (possibly transition stages), or very fine granules, are difficult to recognize.

I have used the dyes employed by Schaffer (1907), especially thionin, in an aqueous solution to stain paraffin sections of the parathyroid glands of the steer, cow, rat, mouse, cat, rabbit, and man. As a counter stain a saturated solution of orange G in oil of cloves was employed. The mast cells with these stains, stand out prominently in blue or purple blue color while the background of connective tissue stains orange or gold color. Transition stages are well defined. The contrast between mast cells and nuclei of the stroma, especially in lymphoid tissue, is well marked by differences in intensity of color. The nuclei of the stroma tissue stains a very faint blue. In deeply stained preparations, the parenchymatous tissue of the parathyroid gland also stains sharply and is well differentiated. The nuclei take on a brilliant light blue or purple color while the cytoplasm stains a faint orange.

METHOD

A large number of fixations may be used. The best results are obtained after fixing in one of the following solutions: Alcoholic formol, Bouin's pieroformol acetic, Regaud's pieroformol, Carnoy's, Zenker's, Flemming's weak and Flemming's strong, bichloride acetic, Orth's and Orth's plus acetic acid. The sections may be cut from 5 to 10 μ. The paraffin is removed by xylol in the usual way. The slides are then washed in 95 per cent alcohol and immersed immediately in tap water for one to two seconds. I omit the graded series of alcohols generally used after the xylol bath without injury to the tissue.

Place the slides in a bath of 1 to 2 per cent solution of thionin for two to three minutes. Wash off excess stain in tap water if light stain is desired. For densely stained, markedly contrasted preparations prolong exposure to



Photographs were made with D and L. Bausch and Lomb camera and Zeiss' lenses. Fig. 1 was made with obj. 16 mm. oc. K8 bellows 42 cm. Fig. 2, obj. 2 mm. oc. K6 bellows 43 cm.

Fig. 1 shows a section of the parathyroid gland of a tuberculous cow. The number of mast cells in the stroma is very large in comparison with that observed in the human material and even larger than the number generally found in the rat or mouse. Fig. 2 represents an enlarged portion of Fig. 1 showing mast cells with distinct granular bodies in the cytoplasm.

thionin from ten to fifteen minutes. Omit washing the slide in water after taking it from the thionin bath. Remove the excess stain from slide about the sections with cloth. Dehydrate by dropping 3 to 5 drops of neutral absolute alcohol over the sections. The sections appear bluish purple to the naked eye. Immerse the slide in a concentrated solution of orange G in oil of cloves. After a minute or two depending on the thickness of the sections and density of the thionin stain, examine preparations under the microscope. Repeat the immersion in the solution of orange G until the thionin stain disappears from the cytoplasm of the connective tissue leaving it a golden orange color. The mast cells are unaffected and remain a deep purple or blue color. Long exposures up to twenty minutes in orange G do not remove the thionin stain of the granules of the mast cells. The parenchymatous tissue, however, becomes faintly orange except the nucleoli of the chief cells which retain a pale light blue coloration. The orange G goes into solution in oil of cloves slowly. This solution should be stirred frequently and allowed to stand for twenty-four hours before using. The solution may be filtered although I have not found this necessary. The solution may be used for a long time before it becomes discolored by the thionin. After removing the slides from the orange G solution, wash thoroughly in xylol and mount in Canada balsam. The thionin may be substituted by methylene blue, toluidine, gentian, and dahlia. More uniform results have been obtained with thionin.

Examination of a section of the parathyroid gland of the cow stained by this method shows that the cytoplasm takes on a faint orange color and only the nuclei of the chief cells retain a light blue stain of the thionin. The nucleoli stain heavier. The mast cells are clear and readily recognizable with low magnification. Under high magnification the nuclei of the mast cells show deeply stained peripherally distributed chromatic bodies. The granular bodies of the cytoplasm retain a deep purplish color. The granules in a single cell are of various sizes and are of a homogeneous consistency. Occasionally the larger granules have a clear central area with a thin peripheral layer of stainable material. In the parathyroid of the cow, I have observed the presence of a small number of mast cells which have very fine granules of uniform size.

SUMMARY OF METHOD

1. Fix material in any of the usual killing agents.
2. Imbed in paraffin.
3. Section material 5 to 10 μ.
4. Remove paraffin in xylol.
5. Remove xylol in 95 per cent alcohol.
6. Rinse in water.
7. Stain in 1 to 2 per cent thionin in water.
8. Remove excess stain in water, remove water about sections, dehydrate.
9. Stain in solution of orange G in oil of cloves.
10. Observe preparation under microscope until desired results are obtained.
11. Wash thoroughly in xylol.
12. Mount in Canada balsam.

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METHOD OF ELIMINATION OF THE ANTICOMPLEMENTARY RESULT IN MANY WASSERMANN TESTS*

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THE occurrence of an anticomplementary result in a Wassermann test is as unsatisfactory to the serologist as it is vague to the clinician, who is often misled into considering such a result as indicating a positive Wassermann. Carefully controlled and improved technic in performing the test and care of the blood sample itself have eliminated a large percentage of such blurred results. However, the drawing and preservation of blood samples from sources outside the laboratory or hospital often cannot be controlled. In addition to the above mentioned ordinary causes of difficulty are those which may be inherent in the serum itself.

Numerous observations have been made upon anticomplementary substances in Wassermann sera, either as a result of direct study, or incidentally in the course of general Wassermann work. A résumé of the literature is not considered essential in this presentation.

The following method of testing anticomplementary sera has been used, under my direction, at the Wesley Memorial Hospital of Chicago for the past three years. The Wassermann technic in general is carried out according to the Cleveland method.† In those tests in which absorption of complement is found in the control, the serum is subjected to a series of dilution tests. In a series of approximately ten tubes, the amount of serum used for each test varies from 0.1 c.c. to 0.005 c.c., with a constant amount of complement and antigen, and a constant hemolytic system. A control is included for each dilution. The amounts of serum used, and the consequent number of tubes, may be varied to the right or left in the type set-up as given, according to the strength of the anticomplementary reaction in the original test. The antigen

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†C. L. Cummer, Manual of Clinical Laboratory Methods, ed. 2, p. 164, 1926, Lea and Febiger.

TYPE SET-UP OF TEST

	1	2	3	4	5	6	7	8	9	10
SERUM (C.C.)	0.1	0.08	0.06	0.05	0.04	0.03	0.02	0.01	0.007	0.005

Remainder of Wassermann test materials constant in amount

RESULTS

Positive										
Test	4	4	4	4	4	4	3	3	2	0
Control	4	4	3	2	1	0	0	0	0	0
Negative										
Test	4	4	4	3	2	1	1	0	0	0
Control	4	4	3	2	2	1	0	0	0	0

dose used is 0.1 c.c. of a 1-50 dilution, per tube, with an anticomplementary unit of 0.1 c.c. of a 1-5 dilution, when used with inactivated negative serum.

Two type results are given. In the positive, the fixation of complement persists in much smaller amounts of serum in the test than in the control. In the negative, there is approximate agreement of lessened complement binding in both test and control with decreasing amounts of serum. The individual results of test and control vary, of course, with every serum, but the curves show the general relationship given in the type results.

In the course of three years in general laboratory work anticomplementary elimination tests have been performed upon various kinds of sera. Sera with rich and varied bacterial flora, some chemically contaminated in ordinary unclean bottles, others showing deterioration after exposure to air, or long standing, or mechanical trauma, these have all been subjected to the given procedure. In addition many have been tested which in the fresh state were anticomplementary from unknown reason.

It is evident that, theoretically, if a weak syphilitic reagin be associated with a strong, nonspecific complement binding substance, the latter may persist while the former is titrated out. Two-plus sera, by the routine test, were inoculated with staphylococcus and incubated one to three days. Strongly anticomplementary sera resulted, which could be titrated down, leaving a definitely positive test and clear control, with a few exceptions. No solution is offered here for these exceptions, or for sera of still lower Wassermann value in which indefinite results were obtained.

It is well known that anticomplementary substances may exert slightly more binding power in the presence of antigen. The strength of this effect is not sufficient, however, to alter titration values significantly, and it does not obscure the reading of results. Both thermolabile and thermostable anticomplementary substances are eliminated by the method.

Conclusion. A method is outlined for the elimination by titration of anticomplementary substances in many Wassermann reactions.

This test was run in conjunction with the Cleveland Wassermann method.

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

CLINICAL AND EXPERIMENTAL

LOBAR PNEUMONIA: A Clinical and Bacteriologic Study of 2000 Typed Cases, Cecil, R. L., Baldwin, H. S., and Larsen, N. P. Arch. Int. Med., 1927, xl, No. 3, p. 253.

A summary of the more important facts derived from this investigation may be stated as follows:

A bacteriologic classification of 2,000 typed cases of lobar pneumonia showed the following distribution of pathogenic organisms: pneumococcus, 95.65 per cent; hemolytic streptococcus, 3.8 per cent; Friedlander's bacillus, 0.4 per cent; influenza bacillus, 0.05 per cent, and Staphylococcus aureus, 0.1 per cent.

The incidence of the various types of pneumococcus was as follows: pneumococcus type I, 33.6 per cent; pneumococcus type II, 19.2 per cent; pneumococcus type III, 13.4 per cent; pneumococcus type IV, 33.1 per cent. There was considerable variation from year to year in the incidence of the various types.

A study of the relation of types to age shows that young people are particularly prone to pneumococcus type I infections, and elderly people to pneumococcus type III. Type II and type IV show no special predilection for any age group.

A study of the incidence of type in relation to sex shows that type I and type II are commoner in men than in women. Type III and type IV are commoner in women than in men. Type III is almost twice as common in women as in men. Type I pneumonia is three times as common in men under 50 as it is in women over 50. On the other hand, type III pneumonia is more than three times as common in women over 50 as it is in men under 50.

The percentage of mixed infections among the cases of pneumococcic pneumonia is lowest in type I cases (18.4 per cent) and highest in the type IV group (31.2 per cent). The incidence of mixed infections varies from year to year, being highest during years when influenza is prevalent. By far the commonest mixed infection was that of the pneumococcus in association with the influenza bacillus. The pneumococcus and hemolytic streptococcus is the next most frequent combination.

The incidence of preexisting systemic disease is highest in the pneumococcus type III group, 63.3 per cent of all type III infections occurring in patients with systemic disease.

The type of pneumococcus appears to bear no relation to the lobe involved or to the extent of the infection. Three and four lobe infections are as relatively frequent in the milder types (I and IV) as in the severe types (II and III).

Twenty-four per cent of the cases of pneumococcic pneumonia showed pneumococci in the blood. The incidence of bacteremia is higher in the severe types (II and III) than in the mild types (I and IV). Type II is essentially the pneumococcus of bacteremia, yielding positive blood cultures in 39.7 per cent of the cases.

No striking differences in the clinical course could be demonstrated as characteristic of the various types of pneumococci. The average duration of the disease for the various types ranged from 8.6 to 9.1 days, type III running the longest course. Type III infections also ran the longest courses in monkeys.

A study of the mode of termination in the cases of pneumococcic pneumonia in which the patients recovered showed that 49.6 per cent terminated by crisis. Crisis was most frequent in type I infections, 56.6 per cent; type II, 50 per cent; type III, 39.3 per cent; type IV, 45.5 per cent.

The death rates for the various types (without specific treatment) were as follows: type I, 20.7 per cent; type II, 42 per cent; type III, 41.6 per cent; type IV, 29.2 per cent. Types I and IV are the milder forms, types II and III are the severe forms, of pneumococcic pneumonia.

A study of the death rate in relation to age shows a steadily increasing death rate for each decade. In patients under 20, the death rate for all types was 11.1 per cent; in those over 60, the death rate was 52.7 per cent. Age is probably the most important single factor in determining the death rate in pneumonia. Pneumococcus type I pneumonia has a low death rate, presumably because it is the pneumonia of young people. Pneumococcus type III pneumonia has a high death rate, chiefly because of its prevalence in elderly people. In patients under 30, type III pneumonia is not a severe infection.

The relation of bacteremia to death rate was striking in all four types. Among 240 patients whose blood remained sterile, the death rate was only 18.7 per cent; among eighty-nine patients whose blood cultures were positive, the death rate was 83.1 per cent.

The association of some systemic disease with pneumonia increases the death rate. Among patients free from systemic disease, the death rate was 23.4 per cent; among those with systemic disease, the death rate was 46.4 per cent.

In all four types of pneumonia the death rate was definitely related to the number of lobes involved; for single lobe infections it was 20.9 per cent; two lobes, 36.3 per cent; three lobes, 40.8 per cent, and four lobes, 65.5 per cent.

Some form of pneumococcic complication occurred in 12.1 per cent of type I cases. The incidence of pneumococcic complications was almost twice as high for type I infections as for the other three types.

Empyema was the commonest complication, occurring in 5.1 per cent of all cases. The incidence of empyema was considerably higher in type I infections than in any of the other types. Empyema is a much more serious complication when it develops during the active stage of pneumonia than when it occurs after the crisis.

VAGINITIS: Clinical Criteria for Differential Diagnosis of Nongonorrheal Infantile Vaginitis: Tomasi, L. Pubelin. Seg. Prat., 1927, xxxiv, 415.

Acute

Gonorrheal:

Acute stage, rather marked, and short. Creamy whitish yellow, or yellow greenish abundant discharge.

Swelling and reddening of external genitals and surroundings.

Involvement of the urethra.

Pus with characteristic intercellular gonococci, gram negative, few epithelial cells and leucocytes.

Fornix, upper vagina involved; erosions, hemorrhagic spots.

Nongonorrheal:

Relative acuteness, short duration. Scarce liquid leucorrheal or mucus, sometimes serous, discharge, with solid and rice water features.

Perigenitals not inflamed, except in uncleanliness.

Urethra not involved.

Fibrin and epithelial cells; more or less pus, cells vacuolized, abundant mixed flora, cocci, bacilli.

Lower vagina involved, no erosions, vivid reddening, hemorrhage diffuse, no gonococci.

Subacute and Chronic

Gonorrheal:

Sudden onset, more or less acute, recurrences.

Pus with intra and extracellular diplococci, gram negative and positive diplococci and bacilli.

Urethritis.

Repetition of acute symptoms.

Repeat examinations in acute stage.

Limited to upper vagina, discharge, hemorrhagic spots, papillomatous appearance of mucosa.

Frequent involvement of cervical canal, pus, mucos, hemorrhage.

Endocervical examination.

Provocation with vaccine brings on recurrence.

Cutireaction and intradermal reaction produce inconsistent results.

Complement deviation in about 50 per cent positive.

Nongonorrheal:

Insidious painless onset, not acute, varying degree of discharge.

Semblance to old gonorrhea, gram test decides.

No urethritis.

Varying acuteness, absence of acute recurrences.

Often scrofulus lymphatic appearance, velvety papules.

Cervical Canal: Often granular aspect, but no discharge.

Provocation with vaccine not effective, local chemical causes slight reactivation.

Cutireaction produces positive Pirquet, or symptoms not in keeping with vaginal condition.

Complement deviation (gonococcal antigen) rare as specific results.

PULMONARY TUBERCULOSIS: The Hemotonic Index and the Resistance of the Red Corpuscles in Pulmonary Tuberculosis, Secco, C. Il Policlinico; July, 1926, xxxiii, 1041.

The hemotonic index is expressed by the equation $\frac{T}{G + Hb}$ in which Hb – hemoglobin; G = red cell count; and T = the maximum arterial pressure in mm.

Normally the index is between 3 and 4.

Secco finds the results of both this index and the red cell fragility test of no prognostic value in tuberculosis.

BLOOD POTASSIUM: A Colorimetric Method for the Determination of Potassium With 0.2 c.c. of Blood, Yoshimatsu, S. Tohoku Jour. Exper. Med., 1926, viii, 174.

Preparation of Reagents

1. Sodium cobalti-nitrite reagent.

Solution A.—25 grams of cobalt nitrate crystals are dissolved in 50 c.c. of water, and to this solution are added 12.5 c.c. of glacial acetic acid.

Solution B.—120 grams of sodium nitrite (potassium-free) are dissolved in 180 c.c. of distilled water. To the whole of solution A are added 210 c.c. of solution B. Air is drawn through the solution until all the nitric oxide gas has passed off. The reagent thus prepared is kept in an ice chest and filtered each time before using. It will keep at least one month.

2. Potassium cobati-nitrite.

Adding slowly 300 c.c. of sodium cobalti-nitrite solution (1) to 50 c.c. of 1 per cent pure potassium chloride, the potassium cobalti-nitrite settle rapidly.

The precipitate is transferred to a Buchner funnel, washed three or four times with cold distilled water until the last washing fails to take color when a drop of ammonium sulphide is added. And then air-dried on the Buchner funnel.

3. Diluted nitric acid.

To 117 c.c. of concentrated nitric acid (spec. w. 1.317) is added 283 c.c. of distilled water.

4. Standard solution.

0.2905 grams of potassium cobalti-nitrite (2) is dissolved in 250 c.c. of diluted nitric acid (3) by warming, then made up to 500 c.c. exactly with distilled water.

5. 1 per cent alcoholic solution of dimethylglyoxim.

6. 1 per cent sodium sulphide solution.
7. Glacial acetic acid.
8. Sodium acetate (powdered).

Procedure

0.2 c.c. of blood is dried in a platinum dish or crucible over the steam bath or in an incubator at 110° C. Then the dish or crucible is placed in a large porcelain dish (6 or 7 cm. in diameter) with pieces of porcelain on the bottom. The porcelain dish and its contents are heated over a low flame until no more fumes come off. Then the porcelain dish with the crucible remaining inside is covered with a quartz or porcelain plate and now heated over a large burner until the blood is completely ashed. The platinum dish or crucible is removed and allowed to cool. The ash is dissolved in 0.5 c.c. of water with one or two drops of glacial acetic acid (7). 0.5 c.c. of sodium cobalti-nitrite reagent (1) is added drop by drop with constant shaking and then allowed to stand for at least 15 minutes. The precipitate is transferred into the centrifuge tube with the aid of a little water, and centrifuged at a speed of 2000 revolutions per minute for 5 minutes.

The supernatant fluid is decanted and 5 c.c. of distilled water poured into the tube, which is centrifuged again. This procedure is repeated three times. After the last washing is decanted, 2 c.c. of distilled nitric acid (3) is added and warmed until the precipitate is dissolved. (Then follows the colorimetric technic below described.)

Direct Determination of Potassium in Serum

According to the technic of Kramer-Tisdall the potassium is precipitated in undiluted serum by adding an excess of sodium cobalti-nitrite reagent.

Details of the Procedure.—1 c.c. of serum is put into a graduated centrifuge tube of 10 or 15 c.c. 2 c.c. of sodium cobalti-nitrite reagent (1) are then added drop by drop with constant stirring. At the end of 45 minutes 2 c.c. of water are added and the contents are again mixed and then centrifuged 15 minutes at a speed of 2000 or 2500 revolutions per minute. The supernatant fluid is decanted by means of centrifugation as above described, and the precipitate washed three times with water. The precipitate is then dissolved in 2 c.c. of diluted nitric acid.

Colorimetric Technic.—1.5 grams of sodium acetate are put into a 50 c.c. volumetric flask and then the potassium cobalti-nitrite from blood or serum dissolved as above mentioned is poured together with the rinsings of the centrifuge tube. 4.0 c.c. of standard solution (4) (2.0 c.c. for serum) are pipetted into another 50 c.c. volumetric flask already containing 1.5 grams of sodium acetate. To both flasks about 20 c.c. of water, 5 c.c. of 1 per cent dimethylglyoxim solution (5) and 2 c.c. of 1 per cent sodium sulphide are added. After 3 minutes both flasks are put in an incubator (110° C.) and kept there for 15 minutes and are then cooled, or they may be dipped into boiling water up to the depth of the neck and kept there for 15 minutes and then cooled in running water. Then water is added up to the mark for colorimetric determination. The colors of the contents of both flasks are compared in a Dubosq colorimeter, the standard being set at 20 mm.

Calculation is as follows:

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} \times 0.4 \times 500 = \text{mg. of K in 100 c.c. blood.}$$

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} \times 0.2 \times 100 = \text{mg. of K in 100 c.c. serum.}$$

LABORATORY TECHNIC

NEUROGLIA FIBERS: A New Method of Staining, Saits, T. La Orienta Bul. Neuro-Biol., 1927, i, 89.

Method A.—Fresh material is fixed in a solution of alum of fluorine (or chrom) 2.5 parts, acetic acid 5 parts, acetic-oxide salt of copper 5 parts and distilled water 98 parts, for one or more weeks. Formalin 5 parts is sometimes added to the solution.

Frozen sections 5 to 10 microns thick are put into a solution of (a) 5 per cent sulphite

of soda 5 c.c., distilled water 98 c.c., and 40 per cent caustic soda 5 drops, or (b) 5 per cent anhydrous sulphate of soda 5 c.c., 5 per cent bisulphate of soda 3 c.c. and distilled water 98 c.c., for 10 minutes. They are then treated with a 0.2 to 0.5 per cent silver nitrate solution in darkness for 1 to 3 days.

The sections are then rinsed and placed in a reducing bath prepared as follows: Two drops of a 40 per cent caustic soda solution are placed in a graduate glass of more than 20 c.c. volume and covered with 3 c.c. of a one per cent silver nitrate solution. A concentrated solution of caustic ammonia is added drop by drop with constant shaking until the precipitate is dissolved. Add another drop of ammonia after complete dissolution. The vessel is then filled up to the 20 c.c. mark. The sections remain in the bath about 15 minutes. As soon as they begin to turn brown, they are transmitted into distilled water and into a solution of equal parts of 20 per cent neutral formalin and distilled water. Within 30 to 45 seconds the sections again become dark brown.

Gilding takes place slowly in a 0.1 per cent gold chloride solution into which a minimum amount of acetic acid may be added. To differentiate the neurofibrils the time of the gold bath is shortened, for the neurofibrils stain bright brown in contrast to the dark green of the glia fibers.

Dehydration and mounting are done as usual.

Counterstain may be done with lithium-carmine, van Gieson fluid, scarlet red or Sudan III.

Method B.—Fresh material is put into a solution of 4 per cent quinine sulphate 45 parts, 4 per cent ammonium bromide 45 parts and formalin 8 parts, for 2 days. Frozen sections are transmitted into the "b" fluid of Method A for 12 hours, rinsed and mordanted in Weigert's routine fluid for from 2 to 4 days. Further treatments follow those of Method A, except that the gilding is omitted since the glia fibers appear dark against the yellowish-brown of the other tissues.

AGGLUTINATION: A Rapid Method for the Macroscopic Agglutination Test, Noble A. Jour. Bact., 1927, xiv, No. 5, p. 287.

For the standard method: One half cubic centimeter suspension plus 0.5 c.c. diluted serum, in agglutination tubes (90 x 8 mm., tapered at the end). Mix well, incubate at 37° C for eighteen to twenty-four hours.

For the rapid method: One tenth cubic centimeter suspension (containing five times as many organisms per cubic centimeter) plus 0.1 c.c. diluted serum (five times more concentrated than for the standard method) in small test tubes (75 by 13 mm.). Shake two minutes and add 0.8 c.c. saline to make the total volume 1 c.c.

Thus we have the same amount of serum and the same number of organisms in each test, for example:

In the standard method: 0.5 c.c. of serum diluted 1:50 contains 0.01 c.c. of undiluted serum.
In the rapid method: 0.1 c.c. of serum diluted 1:10 contains 0.01 c.c. of undiluted serum.

Because the majority are in the habit of thinking of agglutinin titers in terms of dilutions this may be called a dilution of 1:100, meaning that there is actually present 0.01 c.c. of undiluted serum, regardless of the total volume.

Also, in the standard method: 0.5 c.c. of suspension containing 2 x organisms per cubic centimeter = x organisms.

In the rapid method: 0.1 c.c. of suspension containing 10 x organisms per cubic centimeter = x organisms.

The suspension containing 2 x organisms must be of such density as will give maximum agglutination by the standard method with a given organism.

CARBON MONOXIDE: Chemical Tests for This Gas in the Blood, Jones, W. C., and Pinkston, J. O. New Orleans Med. and Surg. Jour., 1927, lxxx, No. 2, p. 99.

Phenol Alone.—1. Undiluted. To undiluted blood in test tubes add 95 per cent phenol in the proportion of 2 to 6 drops to each cubic centimeter of blood and mix thoroughly. Carbon monoxide blood turns a brilliant pink and normal blood, a very markedly contrasting dark chocolate. The immediate results are a little better with the larger proportions of phenol. The colors usually become a little more prominent on standing a few hours to a few days and persist strikingly for many days.

Diluted Phenol Test.—Equal parts of 5 per cent aqueous solution of phenol and undiluted blood yield a very satisfactory result. The CO tube turns a marked red and the normal one, a blackish brown. The reaction is immediate and persists many days to several weeks. The CO blood coagulates incompletely while the normal blood becomes entirely solid. In the tests in which the blood is well diluted either directly or by the addition of diluted reagents, it is well to make the observations both by transmitted light and by light reflected from a white surface, if or as long as the contents remain fluid.

Alum Alone.—To a selected quantity of undiluted blood add an equal amount or one-half as much of a saturated aqueous solution of aluminum potassium sulphate (alum) and mix thoroughly. The blood coagulates, solidifying the contents of both tubes in a few minutes. When the test is first made, the red color of the CO tube is but little different from that of the normal; but on standing a few minutes to an hour, the positive tube turns a cherry red color and the normal one, a dirty blackish brown. The contrast is very marked. Do not expect this test to show positive results at once. It requires a few minutes to an hour for the red of the normal tube to fade sufficiently so that the red in the other tube will stand out by contrast. If, however, a quantity of water is added equal to the amount of blood used, the contrast between the two tubes appears almost at once; but it is not quite as marked as that which is seen ultimately in the tubes with undiluted blood. It is interesting to note that the tubes to which this amount of water is added, solidify as promptly as those without any water (except that which is included in the alum solution).

LYMPH NODES: Method to Obtain Material by Puncture of Lymph Nodes for Study With Supravital and Fixed Stains. Arch. Int. Med., 1927, xl, No. 4, p. 552.

The procedure which has been developed and utilized in studies on lymph nodes is as follows: The skin overlying the structure to be punctured is cleaned surgically by scrubbing with soap, followed by the application of ether and iodine, or by mercuric chloride or picric acid solutions. About 0.5 c.c. of procaine hydrochloride in solution is infiltrated through a small hypodermic needle into the skin. A 17 or 18 gage sterile needle about 3.5 cm. in length is then inserted through the anesthetized area until the point pierces the capsule of the lymph node or diseased tissue. The exact position of the needle point is easily determined by the resistance it encounters and by the movability of the mass. After the needle has been passed just within the tissue from which material is desired, the needle is then steadied and a sterile coarse dental broach is passed through the lumen. These broaches are constructed so that they enter the tissue like a needle but come out like a fish hook, carrying on the barbs numerous elements of living tissue. Smears of the material obtained are then quickly made on glass coverslips. One puncture of a tumor or node yields sufficient material for several smears. The more cellular the tissue the more material is obtained from which cultures as well as smears may be made. After sufficient material has been obtained by inserting one or more broaches, the needle is withdrawn and a small dry sterile dressing applied. The procedure seems to be no more painful than puncturing a vein in the arm for the withdrawal of blood, and there has been no difficulty in making repeated punctures of lymph nodes on the same patients.

Technic of Vital Staining.—A thin film of dye in alcoholic solution is smeared across the surface of a previously cleaned, polished and warmed glass slide. This is done by dipping the end of another slide, with which the smear is made on the former one, into a fresh

mixture (10 drops) of equal parts of 0.5 per cent alcoholic solution of neutral red and a 0.1 per cent alcoholic solution of ianus green. Solutions of the dyes are kept on hand, made up in 95 per cent alcohol. The dyes are not mixed until they are ready for use, because a mixture is not a stable preparation. Solutions of varying concentrations should be employed for making the dye films until one has gained proficiency in estimating the optimum concentration desired. Emphasis should be placed on staining the cells as lightly as is consistent to obtain good differentiation of the structures they contain. If the dyes are employed in concentrated solutions, the nuclei will be stained and the cells quickly killed. The glassware must be scrupulously clean because cells are delicate structures, and any acid, alkali or the like will injure their membranes and markedly alter their physiologic behavior.

Method Modified for Tissue Cells.—In the study of cells from blood, a free-flowing drop of blood is procured on the under surface of a glass coverslip, and this is allowed to fall gently on the slide prepared with the dye. In the case of blood, the plasma takes up some of the dye and acts as a medium in which the cells maintain their activity in suspension. However, material obtained from nodes, especially if fibrosed, often has insufficient fluid to permit cells to be properly suspended. The addition of physiologic sodium chloride alters the staining qualities and behavior of the cells, and is not ideal for this purpose. As a fluid in which to suspend the cells one can use the patient's serum or that from a person belonging to iso-agglutination group I (Moss classification) whose serum does not agglutinate red blood corpuscles. It is essential to have a fluid medium in which to suspend the cells immediately after their removal from the body in order to prevent dessication and to promote a uniform staining reaction.

BLOOD CHEMISTRY: Blood Chloride Methods, Short, J. J., and Gellis, A. D. Jour. Biol. Chem., 1927, lxxiii, No. 1, p. 219.

The objections to several blood chloride methods are pointed out, with special reference to the method of Whitehorn. The end-point in the Whitehorn method is unsatisfactory, as it yields results which usually are too low. The authors' technic, an adaptation of the McLean-Van Slyke iodometric principle to the Folin-Wu tungstic acid filtrate, is described. This is believed to be preferable to the Volhard titration. Tabulated results of a comparison of the methods of Austin and Van Slyke, of Whitehorn, and the proposed method are given. The proposed method possesses the advantages of being short, simple, and accurate.

The method follows:

Sodium Tungstate—A 10 per cent solution.

Sulphuric Acid—a 2/3 N solution.

Silver Nitrate—M/29.25. Dissolve 5.812 mg. of $AgNO_3$ in 600 c.c. of water in a liter flask; add 250 c.c. of HNO_3 (sp. gr. 1.42). Dilute to mark with water.

Potassium Iodide—M/73.1. Dissolve 2.4 mg. of KI in 1 liter of water. Standardize by titrating against 5 c.c. of the $AgNO_3$ solution to which have been added 5 c.c. of water and 6 c.c. of starch indicator. Adjust so that the amount required will be 12.65 c.c. (12.5 c.c. to precipitate the $AgNO_3$ and 0.15 c.c. to develop a definite end-point).

Starch Indicator—(A) Dissolve 2.5 gm. of soluble starch in 100 c.c. of boiling water. Cool and dilute to 150 c.c. (This solution should be prepared fresh each week.) (B) Dissolve 466 gm. of crystalline sodium citrate and 20 gm. of sodium nitrite in about 800 c.c. of water. Dilute to 1350 c.c. Solutions A and B are mixed in necessary quantities before determining chlorides, in the proportion of 1 to 9 respectively.

Procedure

To 1 volume of oxalated blood or plasma (at least 3 c.c.) add 7 volumes of water and mix. Then add 1 volume of the 10 per cent sodium tungstate solution and 1 volume of the 2/3 N H_2SO_4. Shake. After the mixture has changed to a chocolate-brown color, filter. To 20 c.c. of the filtrate in a 50 c.c. centrifuge tube, add 20 c.c. of water, 10 c.c. of the $AgNO_3$ solution; mix by stirring and centrifuge for about 5 minutes in a high powered centrifuge.

Decant through a small filter paper. 20 c.c. of this filtrate are then titrated with the standardized potassium iodide solution, using 6 c.c. of the starch indicator. The end-point is a permanent deep blue color. Calculation: (10.15 c.c. of KI solution used) x 100 = mg. of NaCl in 100 c.c. of blood or plasma.

TUBERCULOSIS: A New Stain for Tubercle Bacilli, Elvers, C. F. Jour. Urol., 1927, xvii, 573.

The film on the slide is made in the usual way, fixed by heat and placed for five minutes in a Coplin jar containing the following stains:

Absolute alcohol	20 c.c.
Melted Phenol Crystals	20 c.c.
Basic Fuchsin	12 grams.
Xylol (C. P.)	90 c.c.

Decolorize by gently rinsing in 0.25 per cent acid alcohol. Rinse for a few seconds in distilled water, air, dry and counter stain with Loeffler's methylene blue.

The results are very satisfactory. The cells and mucosa shreds are not curled or destroyed, and the field is free of all artefacts. The acid-fast bacilli are stained red by the fuchsin; other organisms and cells blue. If this stain be kept at incubator or low oven temperature it will be more penetrating and give greater contrast after several weeks than when freshly made.

SPINAL FLUID: Application of the Combining Power of Proteins With Rose Bengal as a Quantitative Test on the Spinal Fluid, Rosenthal, S. M., and Ackman, F. D. Arch. Int. Med., 1926, xxxviii, 527.

The factors of error can be reduced to a negligible quantity by employing protein dilutions greater than 0.1 per cent. The normal protein content of the spinal fluid is only 0.025 per cent and in disease it rarely increases beyond 1 per cent. In the technic employed the lowest dilution of cerebrospinal fluid is 1:25, which in terms of protein content gives a dilution of 1:100,000 (0.001 per cent) in normal fluids. The following technic has been adopted: 1 c.c. of spinal fluid is diluted to 25 c.c. with distilled water. This is placed in four test tubes as follows:

	Tube 1	Tube 2	Tube 3	Tube 4
Quantity of 1:25 cerebrospinal fluid	10 c.c.	5 c.c.	2.5 c.c.	1 c.c.
Water	0	5 c.c.	7.5 c.c.	9 c.c.

To each tube 1 c.c. of 0.02 per cent rose bengal in aqueous solution should be added, and mixed by inverting the test tubes. To each tube 1 c.c. of tenth normal hydrochloric acid is now added and the solutions again mixed. Comparison of the color which remains should be made in a colorimeter with a standard prepared by adding 1 c.c. of 0.002 per cent rose bengal to 11 c.c. of water.

The P_H of the acidified solutions will be 2.01 to 2.2, at which acidity all of the free rose bengal will be decolorized, so that the color of the solution represents the bound dye. If a colorimeter is not available, a series of standards may be prepared as follows: 10 c.c. of 0.02 per cent rose bengal is added to 110 c.c. of water into which 1 drop of 10 per cent sodium hydroxide has been placed. 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 c.c. of this solution are placed in ten test tubes to which are added 9, 8, 7, 6, 5, 4, 3, 2, 1 and 0 c.c. of faintly alkalized water. They should be tightly corked and preferably sealed with paraffin. Their color will be retained for several months if kept in the dark, but they will rapidly lose color if exposed to the sunlight. These tubes represent from 10 to 100 per cent standards; satisfactory comparisons can be made with them by holding the tubes directly against a sheet of white paper or other white background.

REVIEWS

Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.

How I Came to Be *

A BOOK on human embryology written for the layman who is curious to know the mysteries of the origin and development of the human fetus. The author has added interest by couching the discussion in the form of an autobiographic story, the unborn babe telling in his own way his experiences from day to day, from the time of his first extrusion from the ovary.

It is a difficult subject well handled. Difficult, not from any aspect of prudery, but because even with a good instructor and an intelligent student there are many stages in embryologic development that are not readily visualized. However, while the reader may not be able to follow consecutive development of the various organs step by step, he will easily acquire from the book a fairly good knowledge of what is going on during embryologic development.

Statistical Methods for Research Workers †

THIS is the fifth volume of a series of biologic monographs and manuals the purpose of which is to present an authoritative summary, review and discussion of various fields of biologic investigation.

In many researches the analytic survey of large masses of data by statistical methods is essential and the purpose of the present volume is to present in a relatively simple and clear way the methods whereby such statistical problems may be attacked.

To those required or desiring to use applied mathematics in the analysis of statistical data this volume should be of inestimable value as the subject, necessarily complicated, is presented in a series of clear and logical steps accompanied by numerous tables illustrated and facilitating their practical application.

Food Infections and Food Intoxications ‡

THE purpose of this book is to present in a single volume an authoritative survey of the subject of poisoning and intoxication from food. To this end a large amount of clinical and experimental data scattered throughout the literature has been consulted and discussed.

The resultant volume, apparently the first of its kind, furnishes a very compact, comprehensive, and clear presentation of this very important subject.

The book is divided into three sections, the first, Infections from Food (58 pages),

*How I Came to Be. The Autobiography of an Unborn Infant. By Armenouhie T. Lamson. Pp. 179. Macmillan, New York, 1926.

†Statistical Methods for Research Workers. By R. A. Fisher, Chief Statistician, Rothamsted Experimental Station. Cloth, Second edition, 269 pages, 11 figures, numerous tables. Oliver and Boyd, Edinburgh.

‡Food Infections and Food Intoxications. By S. R. Damon. Associate Professor of Bacteriology, Johns Hopkins University School of Hygiene. Cloth, 266 pages, 33 figures, and 1 plate. Williams and Wilkins Co., Baltimore.

NOTE: In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

discussing paratyphoid in ections from food, tuberculosis from milk and meat, undulant fever from milk, septic sore throat from milk, and actinomycosis. Whenever possible the phases of etiology, symptomatology, diagnosis, treatment and prophylaxis are presented in detail. Under diagnosis, acceptable laboratory methods are described.

The experimental evidence in each type of food poisoning receives due consideration.

Section II (91 pages) is devoted to a discussion of Intoxications from Food, Botulism, mushroom poisoning, and fish and shell-fish poisoning, each being discussed in accordance with the plan above outlined.

In Section III (87 pages) zoo-parasitic infections acquired through food are considered, animal parasites, trichinosis, taeniasis, helmiuth infestation, and other parasites.

The book is deserving of and should receive a warm welcome from physicians and laboratory and public health workers. It is a volume to be purchased rather than borrowed.

*Gonococcal Urethritis in the Male**

SO VOLUMINOUS has the literature upon disease become that while the textbook, as ordinarily understood is needed as a repository for known facts and a comprehensive source of reference, there is a growing, and in many ways a commendable, tendency to resort to the monograph for comprehensive discussions of a single subject or some single phase thereof.

The author of the present volume begins his preface by expressly disclaiming any intention of presenting it as a textbook and classifies it as "a simple story simply told." His intention is to place before his readers the lessons drawn from his experience in the treatment of gonorrhea in the male, and that he has had ample experience, and that it has been profitably digested a perusal of the volume well demonstrates.

He maintains, and with justice in the reviewer's opinion, that gonorrhea is a disease which in the minds of all too many is but little understood, its pathology but ill-appreciated, and the manner of its treatment too many times haphazard.

Dr. Pelouze, then, in a clear and simple manner, discusses the subject from all these angles, with the courage of his convictions and certainly from the standpoint of experience.

In the reviewer's opinion this book could well be read with profit by the practitioner at large and serve as a starting point, at least, for an intelligent method of treatment. It can be highly recommended for the use of those to whom it was written.

Handbook of Histology†

THE purpose of this little volume, to present a short, concise description of tissues and organs with illustrations prepared from class specimens, has been very successfully accomplished. The text is well written and the illustrations carefully selected and prepared. The book should serve a useful purpose.

Aluminum Compounds in Food‡

CONSIDERING the rather bitter controversy which has been and still is being waged by various commercial interests, chiefly regarding the use of baking powders containing aluminum and aluminum cooking utensils, and the many obviously prejudiced publications which have appeared, it is refreshing to review this book by Dr. E. E. Smith which presents a complete digest of the literature upon "Aluminum Compounds in Foods," written with

*Gonococcal Urethritis in the Male. For Practitioners. By P. S. Pelouze, M.D., Associate in Urology and Assistant Genito-Urinary Surgeon at the University of Pennsylvania, 357 pages, 78 illustrations, Cloth. Philadelphia and London: W. B. Saunders Co., 1928.

†A Handbook of Histology. By A. McL. Watson, Lecturer on Histology, University of Glasgow, Cloth, 207 pages, 52 figures, 1 colored plate. W. Wood and Co., New York.

‡Aluminum Compounds in Food. By Ernest Elsworth Smith, Ph.D., M.D. Cloth. Pp. 378. Paul B. Hoeber, Inc., 76 5th Ave., New York. 1928.

the evident purpose of presenting all the known facts. In all except the final chapter in which he endeavors to draw conclusions from this mass of data, Dr. Smith presents the findings and opinions of the various investigators whose work he reviews in an entirely impartial way, often in the authors' words.

The methods of estimating the minute amounts of aluminum present in various animal tissues have been admittedly very unsatisfactory, and the reviewer believes that the figures given in the literature for the somewhat larger amounts of aluminum naturally occurring in foods should be accepted with some reservations.

Since the presence and effects of aluminum compounds in food are of such scientific interest at the present time, it is helpful to have in a single volume all available data on the subject, including a comprehensive résumé of the report of the Referee Board of the U. S. Department of Agriculture, which has not been readily accessible owing to the fact that the report was never printed.

Chapter II gives the natural occurrence of aluminum in food; Chapter III, added aluminum compounds in food; Chapter IV, history and research, 1828-1897; Chapter V, researches of E. E. Smith; Chapter VI, the influence of aluminum compounds on the nutrition and health of man; Chapter VII, litigations relative to baking powders containing salts of aluminum; Chapter VIII, the relation of aluminum compounds on unicellular animal life and on the isolated cells and tissues of higher animals; Chapter X, the solubility in the gastrointestinal tract of aluminum compounds of baking powder residues; Chapter XI, the action in the alimentary tract of food prepared with sodium aluminum sulphate baking powders; Chapter XII, the question of the absorption of aluminum; Chapter XIII, the effects of aluminum compounds when administered subcutaneously or intravenously; Chapter XIV, experimental observations upon the influence of food prepared with baking powder containing sodium aluminum sulphate on the growth and well-being of animals, and Chapter XV, discussion.

The book is an interesting, readable narrative on the subject of aluminum in foods. It is complete, authoritative, and impartial, and will be found very useful to physicians, chemists and others interested in this subject.

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EDITORIALS

The Essential Nature of Pernicious Anemia

IS PERNICIOUS anemia due to an increased destruction of red blood corpuscles or does it result from a disturbance of blood formation? Diverse views have been held by hematologists for the past fifty years. Most workers have considered the evidence for increased blood destruction conclusive, the great excess of bile pigments and other end-products of hemoglobin metabolism in the blood, tissues, and secretions during the clinically active stage of the disease being the principle reason for considering the disease as primarily hemolytic in nature. Some unknown hemolytic agent has been generally accepted as the cause of the disease.

The most characteristic finding at autopsy is the replacement of bone marrow ordinarily filled with fat by hyperplastic red marrow. This marked hyperplasia has been usually interpreted as an attempt to compensate by increased blood formation for the increased blood destruction. The inevitably fatal outcome thus represented the exhaustion of the bone marrow to a point at which it could no longer produce red cells.

Another constant finding at autopsy is the deposit of hemosiderin in the liver, spleen, and kidneys supposedly due to the increased blood destruction. The characteristic clinical and pathologic findings have been correlated to visualize a disease in which there is primarily excessive blood destruction from some toxic agent, followed by a compensatory hyperplasia of the myeloid tissue. If this view were correct, during the remissions of the disease the bone marrow should be forming more cells than during a relapse.

On the other hand some workers have considered the disease as primarily one of the bone marrow. This was the view of Cohenheim who first described in 1876 the characteristic bone marrow findings at autopsy. He considered the disease as due to a disturbance of blood formation. The evidence for increased blood destruction has seemed so overwhelming few have accepted this view. Whipple in 1922[1] suggested that the essential nature of pernicious anemia was a deficiency in stroma formation. He concluded that "pernicious anemia should be looked on as a scarcity of stroma building materials or a disease of the stroma forming cells of the marrow which limits the output of red cell framework."

Until recently the bone marrow was studied only from autopsy material. The findings in the terminal stage were considered as typical of all stages of the disease. A study of the bone marrow changes in material obtained by bone marrow puncture, a procedure introduced by Ghedini in 1908,[2] has yielded information of the greatest value concerning the nature of pernicious anemia. Specimens were first obtained from the bone marrow of patients suffering from pernicious anemia in different stages of the disease by Zadek.[3] He found that the bone marrow returns to normal with a remission. He also noted that with the characteristic megalocytosis of a relapse there is a great increase in the megaloblasts of the bone marrow.

Using the technic of marrow puncture Peabody[4] also greatly extended our knowledge of the changes in the bone marrow during the course of the disease. He showed that the characteristic hyperplasia of the megaloblasts is the more striking the more active the disease and the more profound the relapse. The hyperplasia of the relapse becomes less evident as clinical improvement begins. During a remission only the normal bone marrow reaction may be found.

This development of a megaloblastic hyperplasia in severe relapses and disappearance with the remission is the strongest evidence against a hemolytic disease. It cannot be correlated with the view that increased blood destruction is the primary change followed by a compensatory bone marrow reaction. The findings can be explained only on the supposition that the essential pathologic lesion is in the bone marrow. Peabody concluded that "the course of the anemia would thus appear to be an abnormal type of cell growth consisting in a development of the primitive megaloblast and a failure of differentiation into more mature cells that normally get into the blood." The presence of the excess amount of bilirubin in the blood, tissues, and secretions must still be explained. This probably represents, as Peabody suggests, an excess of pigment over what the bone marrow can use in building red cells in the

active stage of the disease which in fact represents a period in the disease when very few cells are being put out into the circulation. The exceedingly small number of vital staining cells at this period also is corroborative evidence that very few cells are being formed.

The localization of the essential lesion of the disease in the bone marrow does not solve the problem of the etiology. Some toxin of the type long supposed to be the hemolytic agent might conceivably so affect the bone marrow. A lack of stroma building material as suggested by Whipple might produce the same pathologic picture. The results attained by treatment with liver and liver extract throw some light on the question. The liver extract which is equally as potent as liver is free of protein, carbohydrate, iron lipoids, and all known vitamins. It has the chemical characteristics of a nitrogenous base or a polypeptide. Its action is due to a specific maturating effect on the megaloblasts.[5]

We must look on pernicious anemia as due primarily to an inability of the bone marrow to mature red cells resulting from the lack of some specific substance and thus classify it among the deficiency diseases. Tissues other than the liver, such as the kidney, can furnish the specific substance, so the deficiency is not necessarily an hepatic one. It is more likely due to a failure of absorption, or an elaboration in the intestinal tract of the specific maturating substance, perhaps resulting from a long existent achlorhydria, a constant feature of the disease.

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—*R. L. H.*

Experimental Studies in Chronic Lesions of the Lung

SOME of the recent bacteriologic studies in the production of chronic abscess of the lung have revealed information of great interest to the laboratory worker as well as to the clinician. Louis Herrmann, working under Elliott Cutler, in the Laboratory of Surgical Research at Western Reserve, has modified the original Cutler-Schlueter method, and he has been able to produce, at will, chronic abscess of the lung for study over periods of over one hundred days. This modified procedure is dependent upon the interesting fact that anaerobic organisms with spirochetes and fusiform bacilli, obtained from scrapings of pyorrheal sacs, are evidently important factors in the chronicity of lung lesions. Some of these abscesses of the lung have been under study for the past year, and these present all of the clinical and pathologic earmarks of typical chronic human lesions. Vein-segments or blood-clots impregnated with pyorrheal exudate were introduced intravenously and the infected emboli lodging in the lung produced the pulmonary abscesses. Thus, typical pure lesions, uncontaminated by bronchial manipulation, were secured for study.

Intratracheal implantation even with the introduction of a skin-clip was only rarely successful and only when the foreign body remained in the bronchus for two weeks. The intravenous embolic route was, therefore, considered most desirable for experimentation. Kline, Smith, Crowe, and Allen produced lesions by the intratracheal method, apparently because in their experiments the air passages were completely occluded for a sufficient length of time to allow the anaerobes to grow.

The consensus of clinical opinion is that a large proportion of human pulmonary abscesses are the result of aspiration rather than of embolism. The ease with which aspiration can follow anesthetic abolition of the swallowing reflex was pointed out by Ochsner and Nesbitt. These authors criticize severely the stress laid upon the paucity of pathologic data upon which the embolic theory of the origin of pulmonary abscess is based. Aspiration may well be a common means of the production of pulmonary abscess, but embolism can by no means be discarded as a mechanism of the origin of pulmonary abscess. The embolic experimental method, furthermore, is certainly to be preferred to the aspiration method for the study of the pulmonary processes in the dog.

In the study of the factors of chronicity, it was pointed out that Holloway, working in the same laboratory, had shown previously that animals with colon bacillus embolic abscesses had a rising titer of immune bodies in the blood, which resulted in healing of the abscess by cicatrization within three weeks in most animals. The work of Van Allen and of Brown and Archibald on the unfavorable influence of artificially produced cough on the healing of abscess of the lung was repeated. Exposure to diluted (1-3000) chlorine gas for three to five minutes three times each day resulted in attacks of coughing. The increased intrabronchial pressure, incident to coughing, driving the infected material further into the bronchial ramifications and distending the pathologic areas of rarefication in the lung, sufficed to prolong the pulmonary process for not more than five weeks. Cough, therefore, may well be an important mechanical, aggravating factor, but is not alone able to reproduce, experimentally, the chronicity that is the common clinical characteristic of human lung abscess.

Emboli containing the scrapings from pyorrheal sacs were found to reproduce the clinical picture of the lung lesions as they are found in man. The anaerobic organisms, fusiform bacilli, vibrios, and spirochetes are definite factors, therefore, in the chronicity of these lesions. Kline and Smith and Crowe have independently demonstrated that even when not the exciting agent, these organisms as secondary invaders definitely prolong the process. Only 20 per cent of the bronchoscopic implantations were successful, as contrasted to 100 per cent of the hematogenous experiments. It is pointed out that hematogenous metastasis of spirochetal processes has been recorded. The parenchymatous pulmonary abscesses should be differentiated from the bronchitic, or bronchiectasis. The question of whether the process is gangrene rather than abscess has been raised, and it is maintained by some that this differentiation can be made by an examination of the washed sputum.

That the spirochetes are only occasionally the chief factor in these conditions is clinically evident from the rarity of the spectacular therapeutic response of these cases to arsphenamine therapy. In this connection, it is interesting to note that in silver impregnation studies of these tissues of experimental lung abscess, the spirochetes were found in advance of the pyogenic process in the lung parenchyma. The further running down of the anaerobes that are chiefly responsible for the chronicity is being pursued. The reproduction of abscess in fresh dogs from abscesses in previous dogs and from the various reclaimed and isolated organisms from experimental lesions has incriminated the anaerobes, especially, as the chief factors in chronicity.

This experimental work emphasizes the importance of careful clinical laboratory studies and especially bacteriologic examinations of the sputum or expectorated exudate in cases of pulmonary abscess, gangrene, bronchiectasis, and chronic bronchitis.

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